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GABA_A-positive modulator selective discriminative stimulus effects of 1,1,1-trichloroethane vapor

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Abstract

Background—The abuse-related behavioral effects of inhalant vapors are poorly understood but probably involve multiple neurotransmitter receptor mechanisms. The present study examined the receptor systems responsible for transducing the discriminative stimulus of the abused chlorinated hydrocarbon 1,1,1-trichloroethane (TCE) in mice.

Methods—Thirty mice were trained to discriminate 10 min of 12000 ppm TCE vapor exposure from air using an operant procedure. Substitution tests were then conducted with positive GABA_A receptor modulators and/or NMDA receptor antagonists.

Results—The nonselective benzodiazepines midazolam and diazepam produced 62% and 61% and the barbiturate pentobarbital produced 68% TCE-lever selection. Zaleplon, an alpha1 subunit-preferring positive GABA_A receptor benzodiazepine-site positive modulator resulted in 29% TCE-lever selection. The direct extrasynaptic GABA_A agonist gaboxadol (THIP) and the GABA reuptake inhibitor tiagabine failed to substitute for TCE. No substitution was elicited by a competitive (CGS-19755), noncompetitive (dizocilpine) or glycine-site (L701,324) NMDA antagonist. The mixed benzodiazepine/noncompetitive NMDA antagonist anesthetic Telazol and the anticonvulsant valproic acid exhibited low levels of partial substitution for TCE (38% and 39% respectively). Ethanol and nitrous oxide failed to substitute for TCE.

Conclusions—The results suggest that the discriminative stimulus effects of TCE are fairly selectively mediated by positive modulation of GABA_A receptors. The failure of gaboxadol to substitute and the poor substitution by zaleplon suggests that extrasynaptic GABA_A receptors as well as GABA_A receptors containing alpha 1 subunits and are not involved in transducing the discriminative stimulus of TCE. Studies with additional GABA_A benzodiazepine-site positive modulators will be necessary to confirm and extend these findings.

Keywords

inhalants; drug discrimination; 1,1,1-trichloroethane; solvents

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1. Introduction

Abuse of volatile inhalants is a serious problem worldwide. The incidence of inhalant abuse in the United States exceeds that for many other more widely publicized drug classes (DHHS, 2010). In particular, children and adolescents are uniquely susceptible to inhalant abuse perhaps due to the presence of abusable inhalants in a variety of common household products including motor fuels, cleaning products and paints, among others (Johnston et al., 2011). Despite the seriousness of the inhalant abuse problem, research examining the neurochemical systems affected by inhalants has lagged behind that for virtually all other classes of abused drugs. One serious impediment to this line of inquiry is that dozens, if not hundreds, of different compounds from a number of chemical classes have been reported to be abused by inhalation, making categorization and prioritization a challenge.

One major class of abused inhalants is the volatile hydrocarbons. This large grouping of compounds can be further subdivided by chemical structure. Prominent among the volatile hydrocarbons, due to their demonstrated abuse liability, are the chlorinated hydrocarbons which includes compounds such as 1,1,1-trichloroethane (TCE), trichloroethylene and perchloroethylene (Bowen et al., 1999a; Flanagan et al., 1990; Troutman, 1988). Although relatively few experiments have been conducted exploring the neurochemical mechanisms of chlorinated hydrocarbons, the overarching finding has thus far been that they have *in vitro* effects on several receptors. Specifically, TCE and trichloroethylene positively modulate GABA_A and glycine receptors expressed in *Xenopus* oocytes (Beckstead et al., 2001; Beckstead et al., 2002; Beckstead et al., 2000). TCE and trichloroethylene enhance GABA-mediated synaptic inhibition in rat CA1 pyramidal neurons (MacIver 2009). TCE also attenuates NMDA-induced currents (Cruz et al., 2000) as well as enhances 5-HT₃ receptor function in recombinant receptors expressed in *Xenopus* oocytes (Lopreato et al., 2003). Perchloroethylene inhibits nicotinic acetylcholine receptors expressed in oocytes (Bale et al., 2005). Finally, acute exposure to TCE decreases DAMGO binding to mu-opioid receptors in discrete brain regions (Paez-Martinez et al., 2008). While these studies demonstrate that exposure to chlorinated hydrocarbons can affect neurotransmitters in isolated systems, which, if any, of these mechanisms are responsible for generating the abuse-related behavioral effects of inhalants such as intoxication are uncertain.

All drugs of abuse produce subjective states of intoxication. These subjective stimulus effects can be modeled in animals using the drug discrimination paradigm (Balster, 1991; Colpaert, 1999; Schuster and Johanson, 1988). In drug discrimination, animals are trained to attend to the presence or absence of the internal state produced by an experimenter-administered training drug. This is accomplished by reinforcing only drug-appropriate behavior (usually an operant response) after training drug administration and only reinforcing vehicle-appropriate behavior following vehicle administration. One major advantage of drug discrimination is its neurochemical specificity. In general, only test drugs that act through neurochemical actions similar to the training drug will produce training drug-appropriate responding. Test drugs that produce their effects via other receptor mechanisms will elicit only vehicle-appropriate responding even at doses that produce profound behavioral effects (Colpaert, 1999).

Drug discrimination has been used extensively to examine the subjective stimulus produced by most classes of abused drugs. However, very few studies have examined chlorinated hydrocarbons either as training drugs themselves or as cross test drugs in animals trained to discriminate other compounds. Specifically, TCE produced partial substitution in mice trained to discriminate ethanol from saline (Rees et al., 1987b) or diazepam from saline (Bowen et al., 1999b). TCE produced partial to full substitution in mice trained to discriminate pentobarbital from vehicle (Rees et al., 1987a). In contrast TCE failed to

substitute in mice trained to discriminate the noncompetitive NMDA antagonists, phencyclidine or dizocilpine (MK-801) from vehicle (Bowen et al., 1999b; Shelton and Balster, 2004). Lastly, TCE produced full substitution in mice trained to discriminate isoflurane vapor from air (Shelton and Nicholson, 2010).

Only two studies, both from our laboratory, have trained TCE as a discriminative stimulus. We demonstrated that there is substantial overlap in the discriminative stimulus effects of TCE vapor with other chlorinated hydrocarbons, aromatic hydrocarbons and halogenated vapor anesthetics (Shelton, 2009). In the second experiment, we showed that the nonselective benzodiazepine, midazolam, produced a high level of partial substitution in TCE vapor-trained mice but that benzodiazepine-site antagonist, flumazenil, did not attenuate the discriminative stimulus of TCE (Shelton, 2010). We also showed that the noncompetitive NMDA antagonists, PCP and dizocilpine did not substitute for TCE. Lastly, we demonstrated through both agonist and antagonist testing that neither the nicotinic acetylcholine nor the mu opioid receptor systems appear to be involved in producing the discriminative stimulus effects of TCE. When combined, these experiments suggest that the discriminative stimulus effects of chlorinated hydrocarbon such as TCE may be due, at least partially, to their GABA_A positive modulatory effects. However, this hypothesis is tentative given that it is based on positive substitution data from a single positive GABA_A modulator.

The first goal of the present study was to expand on our prior results by examining other classes of GABA_A positive modulators in TCE-trained mice. Further, we wished to more thoroughly explore whether NMDA antagonist effects may be responsible for transducing TCE's discriminative stimulus by examining drugs acting upon additional modulatory sites on the NMDA receptor. Lastly, we examined several additional compounds that are believed to modulate either GABA_A and/or NMDA receptor activity.

In addition to examining the neurochemical specificity of TCE, we also wished to quantify the rate of onset and offset of TCE's discriminative stimulus effects. As virtually no work has been conducted examining the pattern of human inhalant use within a single binge, we hypothesized that onset and offset of discriminative stimulus effects in animals could be a useful predictor of intra-episode abuse patterns in humans. For instance, inhalants with relatively slow onset and offset of discriminative stimulus effects might be expected to promote fewer but more extended periods of exposure during a binge given that longer exposure would be required to produce pharmacologically meaningful effects. In contrast, inhalants with rapid onset and offset of stimulus effects might be predicted to result in shorter but more frequent inhalation episodes within a binge.

2. Methods

2.1 Subjects

Thirty adult male B6SJLF1/J mice (Jackson Laboratory, Bar Harbor, Maine) served as subjects. The B6SJLF1/J strain has been used extensively in our laboratory for prior inhalant drug discrimination studies (Shelton, 2007; Shelton, 2009; Shelton and Slavova-Hernandez, 2009; Shelton, 2010). The mice were individually housed on a 12 hr light/dark cycle (lights on 7 AM). Feeding was adjusted to maintain a healthy, stable weight of between 27–35g for the duration of the study. These studies were conducted in accordance with the Institute of Laboratory Animal Research Guide for the Care and Use of Laboratory Animals. The studies were reviewed and approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

2.2 Compounds

99.5% anhydrous 1,1,1-trichloroethane (TCE), dizocilpine maleate (MK-801), tiagabine hydrochloride, valproic acid and gaboxadol hydrochloride (THIP) were purchased from Sigma-Aldrich Chemicals (Milwaukee, WI). mCPP (m-chlorophenylpiperazine) hydrochloride and zaleplon were purchased from Tocris Bioscience (Ellisville, MO). L701,324 (7-Chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(1H)-quinolinone) was obtained from the NIDA Drug Supply Program (Bethesda, MD). Midazolam maleate was a gift of Roche Pharmaceuticals (Nutley, NJ). Telazol (equal concentrations of tiletamine and zolazepam), pentobarbital and diazepam were purchased as injectable solutions from Webster Veterinary Supply (Charlotte, NC). 95% ethanol was purchased from Pharmco-Aaper (Brookfield, CT). Nitrous oxide gas (USP) was purchased from National Welders (Richmond, VA). Injected drug doses were based on their total weights. Dizocilpine, tiagabine, valproic acid, gaboxadol, midazolam, pentobarbital, ethanol, Telazol and mCPP were prepared in 0.9% physiological saline. Diazepam and zaleplon were prepared in a solution of 45% hydroxypropyl-beta-cyclodextrin (Acros Organics) in sterile water. L701,324 was prepared as a suspension in 10% cremophor in sterile water. Midazolam, pentobarbital, dizocilpine, Telazol and ethanol were injected 10 min pre-session. Diazepam was injected 15 min pre-session. Valproic acid, L701-324, mCPP, tiagabine, CGS-19755, gaboxadol, mCPP and zaleplon were injected 30 min pre-session. All injected drugs were administered intraperitoneally (i.p.) at a volume of 10 ml/kg.

2.3 Apparatus

Drug discrimination sessions were conducted in standard 2-lever mouse operant conditioning chambers equipped with 0.01 ml liquid dippers and housed in sound attenuating cubicles (Med Associates model ENV-307AW, St. Albans, VT). Drug discrimination schedule conditions and data recording were controlled by a Med Associates interface and Med-PC version 4 software (Med Associates, St. Albans, VT). The milk solution reinforcer consisted of 25% sugar, 25% nonfat powdered milk and 50% tap water (by volume).

The static vapor chambers and general procedures used to expose the mice to TCE vapor prior to drug discrimination testing have been previously described (Shelton 2007). Closed-loop recirculation of chamber atmosphere through a Miran 1A single wavelength infrared spectrophotometer indicated that TCE vapor concentration in the chambers reached equilibrium in less than 1 min for all tested TCE concentrations (as described in Shelton, 2007). The dynamic system used to expose mice to nitrous oxide has also been described previously (Shelton and Nicholson, 2010).

2.4 Discrimination Training

Daily 15-min training sessions were conducted 5 days per week (M-F). Both lever lights and the houselight were illuminated for the duration of the session. Responding was initially reinforced on only one of the two levers on a fixed ratio 1 response (FR1) schedule for several daily sessions. Upon completion of the FR requirement, the dipper cup was available for 3 sec. Responses occurring while the dipper was elevated did not count toward completion of the next FR. Once the animals were reliably responding at FR1 on either lever, the operant session length was decreased to 5 min and discrimination training was initiated. During each 5-min TCE or air-discrimination training session the correct lever was determined by whether the subject received a 10-min exposure to 12000 ppm TCE vapor or air immediately before the discrimination training session. Training exposures were presented according to a double alternation schedule (i.e. two TCE vapor days followed by two air days). Over the course of 10–20 sessions, the response requirement was increased to FR12. Responding on the inactive lever reset the FR requirement on the correct lever. These

training conditions were in effect for the remainder of the study. Animals were determined to have acquired the 12000 ppm TCE vapor versus air discrimination when the first FR was completed on the correct lever, prior to the completion of a FR on the incorrect lever, in 8 out of 10 consecutive training sessions. Additionally, the mice were required to emit greater than 80% of total responses on the correct lever during all 10 of these sessions.

2.5 Substitution Test Procedure

Following acquisition, substitution tests were conducted on Tues and Fri, providing that the mice continued to exhibit accurate stimulus control on the Mon, Wed, and Thurs training sessions. Test sessions were temporarily suspended if an animal did not emit the first FR on the correct lever and produce greater than 80% correct-lever responding during all training sessions since the last test session. Substitution tests with TCE were preceded by a 5, 10 or 20 min exposure to a single concentration of TCE vapor in the exposure chamber. Substitution tests with nitrous oxide were preceded by a 20 min exposure to nitrous oxide combined with oxygen. In substitution test sessions with injected drugs, the injection was given and the animals were then exposed to air for 10 min in the vapor exposure chamber before being removed and tested for substitution.

Drug discrimination test sessions were 5 min in duration and completion of the FR requirement on either lever resulted in dipper presentation. Inhalant concentrations and drug doses were generally administered in an ascending order. Prior to each substitution curve, two control substitution test sessions were conducted, one following 10 min of exposure to 12000 ppm TCE vapor and a second after 10 min of air exposure. Control sessions prior to the dose-effect curves using injected drugs were conducted in a similar manner with the addition of injection of the test drug's vehicle before air exposure.

2.6 Drug Discrimination Data Analysis

Percentage TCE-lever responding and response rates (responses/sec) were recorded in 30-sec bins. A prior study with TCE from our laboratory indicated that the response-rate altering effects of TCE vapor are extremely short-lived (Shelton, 2010). Therefore, to insure that the discrimination and operant rate suppression data represented a time frame during which the maximal behavioral effects of TCE were exhibited, only the first min of each TCE vapor discrimination test session was examined. To maintain consistency across the entire study, only the first-min data was examined for injected compounds as well. Group means ($\pm$ SEM) were calculated for percentage first-min TCE-lever selection and first-min response rate. Any test dose or concentration that suppressed response rates to the extent that the animal did not complete at least one FR during the first min of the test session resulted in the exclusion of that mouse's datum from the group lever-selection analysis, although that animal's datum was included in the response rate determination. A criterion of 80% or greater mean TCE vapor-appropriate responding was selected to indicate full substitution for the TCE training concentration. Mean TCE vapor-lever responding between 20% and 79% was defined as partial substitution. Mean TCE vapor-lever responding of less than 20% was considered evidence of no substitution. When possible EC_{50} or ED_{50} values (and 95% confidence limits) for TCE vapor-lever selection and response rate suppression were calculated using a Microsoft Excel spreadsheet based on SAS Pharm/PCS version 4 (Tallarida and Murray, 1986). In order to examine the effect of differing TCE exposure durations (5, 10 or 20 min), pair-wise substitution EC_{50} potency ratios with 95% confidence limits were also calculated. If in each of these comparisons the upper and lower potency ratio 95% confidence limits did not encompass a value of 1, the EC_{50} 's between the two exposure durations compared were considered significantly different (Colquhoun 1971).

3. Results

All 30 mice were tested to determine the 10 min TCE exposure concentration-effect curve. TCE vapor concentration-dependently substituted for the 12000 ppm TCE vapor training exposure (Figure 1, upper panel). Full substitution was generated by exposure to TCE concentrations of 8000 ppm and greater. The EC₅₀ for TCE substitution was 2560 ppm (CL: 2240-2927 ppm). Ten minutes of TCE vapor exposure also concentration-dependently suppressed operant responding during the first min of the test sessions (Figure 1, lower panel). The EC₅₀ for response rate suppression was 19560 ppm (CL: 17655-21670 ppm).

Figure 2 shows substitution data following exposure to 5, 10 or 20 min of increasing concentrations of TCE vapor. Concentration-dependent full substitution was generated at each of the three TCE exposure durations. The potency of TCE following 10 min of exposure was not significantly different from that following 20 min of exposure [EC₅₀ potency ratio 1.04:1 (CL: 0.61–1.78)]. However, the potency of TCE following 5 min of exposure was significantly ($p < 0.05$) lower than following 20 min of exposure [EC₅₀ potency ratio 1.64:1 (CL: 1.10–2.54)]. Likewise, the potency of TCE following 5 min of exposure was significantly lower than that following 10 min of exposure [EC₅₀ potency ratio 1.61:1 (CL: 1.05–2.6)].

Figure 3 (upper panel) depicts substitution data resulting from injections of different doses of midazolam, diazepam and zaleplon. The nonselective benzodiazepine site positive modulator, midazolam (circles) produced dose-dependent partial substitution for the 12000 ppm TCE training condition. A maximum of 62% TCE-lever selection was produced by doses of both 10 and 15.6 mg/kg midazolam. The ED₅₀ for midazolam substitution for TCE was 4.5 mg/kg (CL: 2.8–7.0 mg/kg). Midazolam also produced a modest dose-dependent suppression of responding (lower panel) with the highest dose, 30 mg/kg, producing a 45% suppression of responding compared to vehicle control. Likewise, another nonselective benzodiazepine, diazepam (squares) produced dose-dependent partial substitution for TCE with a maximum of 61% TCE-lever selection at a dose of 17 mg/kg. The substitution and response-rate suppression ED₅₀ values for diazepam were 14.9 mg/kg (CL: 3.4–65.4 mg/kg) and 17.9 mg/kg (CL: 12.7–25.1 mg/kg), respectively. Zaleplon (triangles), which exhibits selectivity for GABA_A receptors containing $\alpha 1$ subunits, elicited little substitution for TCE. Maximal substitution of 28% TCE-lever selection was produced at the highest dose (5.6 mg/kg) of zaleplon. Zaleplon dose-dependently suppressed operant responding (lower panel) with an ED₅₀ of 4.0 mg/kg (CL: 2.3–7.1 mg/kg).

Table 1 shows the level of TCE substitution and response rate suppression produced by pentobarbital, valproic acid, tiagabine, gaboxadol and ethanol. The short acting, GABA_A receptor barbiturate-site positive modulator, pentobarbital also resulted in dose-dependent partial substitution for 12000 ppm TCE with an ED₅₀ of 24.3 mg/kg (CL: 20.9–28.1 mg/kg). A maximum of 68% TCE-lever selection was produced by the 30 mg/kg pentobarbital dose. Pentobarbital dose-dependently suppressed operant responding with an ED₅₀ of 24.3 mg/kg (CL: 20.9–28.1 mg/kg). The anticonvulsant valproic acid produced a maximum of 39% TCE-lever responding at the 560 mg/kg dose. Valproic acid generated a response rate suppression ED₅₀ of 460 mg/kg (CL: 341–619 mg/kg) and almost completely suppressed responding at 720 mg/kg. The GABA uptake inhibitor tiagabine failed to substitute for TCE. The response rate suppression ED₅₀ of tiagabine was 3.2 mg/kg (CL: 2.2–4.7 mg/kg) and response rates were almost abolished at 10 mg/kg. The direct extrasynaptic GABA_A receptor agonist, gaboxadol also markedly suppressed responding with an ED₅₀ of 2.4 mg/kg (CL: 1.0–5.7 mg/kg) but failed to produce greater than air control levels of TCE-lever selection. Ethanol produced a maximum of 23% TCE-lever selection at a dose of 2000 mg/kg.

Table 2 shows the level of TCE substitution and response rate suppression produced by drugs representing three classes of NMDA antagonists as well as nitrous oxide and Telazol. The channel blocking NMDA antagonist, dizocilpine, suppressed operant responding with an ED₅₀ of 0.44 mg/kg (CL: 0.37–0.53 mg/kg) but failed to substitute for TCE across doses of 0.03 to 0.56 mg/kg. Likewise the competitive NMDA antagonist, CGS-19755 suppressed operant responding with an ED₅₀ of 11.8 mg/kg (CL: 9.5–14.9 mg/kg) but produced no greater than 25% TCE-lever responding. Lastly, the glycine-site NMDA antagonist, L701,324 did not produce any TCE lever responding across a dose range of 1–56 mg/kg, but it also exhibited only modest response-rate suppressing effects up to a 56 mg/kg dose which was the upper dose limit due to the solubility of the compound. Nitrous oxide failed to substitute for TCE up to a 75% concentration, which was near the upper limit of that which could be administered in combination with 100% oxygen without concern of hypoxia. At 75%, nitrous oxide response rates were modestly suppressed to 44% of the oxygen control response rate. The mixed benzodiazepine/channel blocking NMDA antagonist anesthetic preparation Telazol produced partial substitution with a maximum of 38% TCE-lever responding at the 30 mg/kg dose. Telazol suppressed operant responding with an ED₅₀ of 27.2 mg/kg (CL: 22.9–33.4 mg/kg) and completely abolished operant responding in all animals tested at 56 mg/kg.

4. Discussion

Since the speed of onset of drug effects as well as duration of action are important factors controlling the pattern of drug use we wished to further explore the onset and offset of TCE's discriminative stimulus. We have previously demonstrated that the discriminative stimulus effects of 10 min of exposure to TCE vapor as well as TCE blood levels decline rapidly following the cessation of exposure (Shelton, 2010). The present study explored the onset kinetics of TCE's discriminative stimulus. The TCE substitution concentration-effect curve conducted at the 5 min exposure duration produced a significantly lower TCE potency compared to the substitution curve conducted at the 10 min exposure used for training (Figure 2). In contrast, doubling the duration of TCE exposure to 20 min did not produce a concentration-effect curve significantly different from the 10 min exposure duration. These data suggests that maximal discriminative stimulus effects of 12000 ppm TCE are achieved rapidly in between 5 and 10 min of exposure.

While examining the *in vivo* kinetics of inhalants such as TCE are important, the primary goal of the present study was to more fully explore the neurochemical systems underlying the discriminative stimulus effects of TCE. We have previously demonstrated that the short-acting GABA_A benzodiazepine-site positive modulator midazolam produced partial substitution for 12000 ppm TCE vapor (Shelton, 2010). These results are in agreement with data showing that TCE acts as a positive GABA_A modulator in recombinant receptors expressed in oocytes (Beckstead et al., 2000). In the present study we replicated our prior results, and demonstrated a virtually identical degree of substitution with a second benzodiazepine, diazepam (Figure 3). In addition, we demonstrated that the barbiturate, pentobarbital, produces a similar level of substitution as diazepam and midazolam (Table 1). The GABA_A receptor chloride ionophore is a heteropentamer composed of 5 subunits. Alpha subunit composition is critical for benzodiazepine sensitivity. Classical benzodiazepines such as midazolam and diazepam are fairly nonselective, showing equally high efficacy at GABA_A receptors containing alpha 1,2,3 and 5 subunits but no appreciable affinity at physiologically relevant concentrations for alpha 4 and 6 containing receptors (Harris et al., 1997). Likewise, barbiturates are fairly nonselective positive allosteric modulation of GABA_A receptors (Harris et al., 1997; Pistis et al., 1997; Rahman et al., 2006). These results indicate that GABA_A positive modulation is important for the discriminative stimulus effects of TCE, but do not suggest which subunits are involved.

The primary mediator of benzodiazepines discriminative stimulus effects appear to be the alpha 1 subunit, although other alpha subunits may also be involved (Licata et al. 2005). Therefore, we examined substitution of the alpha 1 preferring benzodiazepine-site positive modulator, zaleplon, for TCE (Rowlett et al., 2000). Although the selectivity of zaleplon for alpha 1 containing GABA_A receptors is fairly modest, zaleplon failed to produce more than a low level of partial substitution for TCE. This was despite testing zaleplon at doses that produced response rate decreases comparable to doses of midazolam and diazepam that produced much greater substitution (Figure 3). These findings suggest that positive modulation of alpha 1 subunit-containing GABA_A receptors may not be involved in producing the discriminative stimulus of TCE.

While positive GABA_A modulation by benzodiazepines and barbiturates is sufficient to elicit TCE-like discriminative stimulus effects, other drugs which enhance GABA_A receptor function do not substitute for TCE. The GAT-1 GABA transporter inhibitor tiagabine failed to substitute for TCE (Table 2). Likewise, the extrasynaptic GABA_A receptor agonist gaboxadol which is thought to preferentially bind to GABA_A receptors containing alpha 4, alpha 6 and delta subunits (Chandra et al., 2006; Herd et al., 2009; Saarelainen et al., 2008) also failed to substitute for TCE. This latter outcome may not be surprising given that benzodiazepines do not substitute for gaboxadol (Michelsen et al., 2007). Somewhat more surprising, however, is the failure of ethanol to substitute for TCE despite numerous studies indicating that ethanol has both barbiturate and benzodiazepine-like discriminative stimulus effects (Grant and Colombo, 1993; Sanger, 1993; Shelton and Balster, 1994). This lack of substitution may be due to non-GABAergic components of ethanol's mixed discriminative stimulus masking its GABA_A-positive modulatory effects and are similar to data showing that ethanol doesn't substitute in animals trained to discriminate benzodiazepines (Ator and Griffiths, 1997; Shannon and Herling, 1983). Valproic acid which among a host of effects acts to inhibit GABA transaminase (Johannessen, 2000) showed modest partial substitution for TCE. Interestingly, valproic acid produced almost full substitution in mice trained to discriminate the vapor anesthetic isoflurane from air and TCE itself fully substituted for isoflurane (Shelton and Nicholson, 2010). These overlapping discriminative stimulus effects suggest that chlorinated hydrocarbons and halogenated volatile anesthetics may be functioning through similar mechanisms.

TCE is an NMDA receptor antagonist *in vitro* (Cruz et al., 2000). However, in mice trained to discriminate TCE from air, the noncompetitive NMDA antagonists, dizocilpine and PCP did not substitute for TCE (Shelton, 2010) nor did TCE substitute in mice trained to discriminate either PCP or dizocilpine from vehicle (Bowen et al., 1999b; Shelton and Balster, 2004). The NMDA receptor complex can also be negatively modulated by competitive antagonists as well as by drugs acting upon the glycine co-agonist site (Bennett et al., 1990; Priestley et al., 1996). Given that site-selective NMDA antagonists can be discriminated from each other (Bobelis and Balster, 1993; Nicholson and Balster, 2009; Willetts et al., 1989) we speculated that our previous negative substitution results with dizocilpine and PCP might have been due to only probing for channel blocking NMDA antagonist-like effects of TCE. This does not, however, appear to be the case given that in addition to dizocilpine neither CGS-19755, nor L701,324 produced any appreciable degree of substitution for TCE (Table 2).

We also examined the possibility that an NMDA antagonist might augment the substitution of a benzodiazepine by testing Telazol which is a 50/50 combination of the channel blocking NMDA antagonist, tiletamine and the nonselective benzodiazepine, zolazepam. Telazol substitution was less robust than that produced by midazolam or diazepam (Table 1). This outcome suggests that rather than augmenting the TCE-like discriminative stimulus effects of a benzodiazepine, co-administration of a channel blocking NMDA antagonist was at best

ineffective and potentially even reduced the ability of zolazepam to substitute for TCE. However, as we could not procure a source of zolazepam we could not confirm this latter hypothesis.

In summary, although TCE has *in vitro* effects upon a number of neurotransmitter receptors, current and previous experiments suggest that a large proportion of the discriminative stimulus of TCE is the result of positive modulation of GABA_A receptors. However, given that none of our test compounds produced full substitution for TCE, other mechanisms not yet examined may also be involved. The failure of zaleplon to substitute for TCE implies that TCE may not interact with the GABA_A alpha 1 subunit. Likewise the failure of gaboxadol to substitute for TCE suggests that alpha 4, alpha 6 and delta containing GABA_A receptors may not be involved in producing TCE's discriminative stimulus effects. Since both barbiturates and classical benzodiazepines have effects on GABA_A receptors containing the alpha 2, 3 and 5 subunits, examination of compounds selective for these subunits in TCE discriminating animals is warranted. The alpha 5 subunit is a particularly interesting target given evidence that alpha 5 subunits may play a role in benzodiazepine sedation (Savic et al., 2008) an effect, which is also a prominent feature of TCE exposure (Bowen and Balster, 1996).

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Fig. 1.

Concentration-effect curve for inhaled 1,1,1-trichloroethane (TCE) vapor in mice trained to discriminate 10 min of exposure to 12000 ppm inhaled TCE from air ($n=30$). The data presented are based on the first min of each 5-min test session. Points above Air and TCE represent the results from air (circle) and 12000 ppm inhaled TCE (square) exposure control sessions. Mean ($\pm$ SEM) percentages TCE-lever responding are shown in the upper panel. Mean ($\pm$ SEM) response rates in responses/sec are shown in the bottom panel. Numbers in parentheses indicate the number of animals out of the group that emitted sufficient responses to be included in the percentage TCE-lever selection curve at a given concentration.

Fig. 2.

Concentration-effect curve for 5 min (circles), 10 min (squares) or 20 min (triangles) of inhaled 1,1,1-trichloroethane (TCE) vapor in mice trained to discriminate 10 min of exposure to 12000 ppm inhaled TCE from air ($n=30$). The data presented are mean ($\pm$ SEM) percentages TCE-lever responding based on the first min of each 5-min test session. Points above Air and TCE represent the results from air (open symbols) and 12000 ppm inhaled TCE (filled symbols) exposure control sessions.

Fig. 3.

Dose-effect curves for midazolam (circles), diazepam (squares) and zaleplon (triangles) in mice trained to discriminate 12000 ppm inhaled TCE vapor from air (n=9–12). The data presented are based on the first min of each 5-min test session. Points above Air and TCE represent the results from air (open symbols) and 12000 ppm inhaled TCE (filled symbols) exposure control sessions. Mean ($\pm$ SEM) percentages TCE-lever responding are shown in the upper panel, mean ($\pm$ SEM) response rates in responses/sec are shown in the bottom panel. Numbers in parenthesis indicate the number of animals out of the group that emitted sufficient responses to be included in the percentage TCE-lever selection curve at a given dose.

Table 1

1,1,1-trichloroethane (TCE) lever selection and operant response rates produced by pentobarbital (n=10), valproic acid (n=7), tiagabine (n=8), gaboxadol (n=8) and ethanol (n=8). Numerals in brackets [] in the % TCE-lever responding column indicate number of animals in the group that emitted sufficient responses to be included in this determination.

Drug	Drug dose or vapor concentration	% TCE-lever responding ($\pm$ SEM)	Response rate in responses/sec ($\pm$ SEM)
Pentobarbital	Air + vehicle	0.5 (0.3)	1.2 (0.2)
	12000 ppm TCE	99 (0.3)	1 (0.1)
	1 mg/kg	8 (0.3)	1.1 (0.1)
	3 mg/kg	1 (0.1)	1.3 (0.2)
	10 mg/kg	3 (2.7)	1.3 (0.2)
	17 mg/kg	17 (7.2)	0.9 (0.1)
	25 mg/kg	52 (10.9) [8/10]	0.4 (0.1)
	30 mg/kg	68 (13.5) [8/10]	0.5 (0.1)
Valproic acid	Air + vehicle	0.2 (0.2)	1.2 (0.3)
	12000 ppm TCE	97 (1.0)	1 (0.1)
	100 mg/kg	4 (2.7)	1.3 (0.2)
	300 mg/kg	4 (3.3)	1 (0.2)
	560 mg/kg	39 (15.5)	0.5 (0.1)
	720 mg/kg	-	0.1 (0.1)
Tiagabine	Air + vehicle	1 (0.4)	1.4 (0.1)
	12000 ppm TCE	85 (12.1)	1.3 (0.2)
	1 mg/kg	1 (0.9)	1.2 (0.2)
	1.7 mg/kg	1 (0.9)	1.2 (0.2)
	3 mg/kg	11 (10.6)	1 (0.2)
	10 mg/kg	-	0 (0)
Gaboxadol (THIP)	Air + vehicle	13 (12.4)	0.9 (0.2)
	12000 ppm TCE	95 (4.9)	1.1 (0.2)
	0.3 mg/kg	2 (1.4)	0.9 (0.2)
	1 mg/kg	2 (1)	0.7 (0.2)
	3 mg/kg	6 (2.5)	0.8 (0.2)
	10 mg/kg	1 (0.5)	0.1 (0.1)
Ethanol	Air + vehicle	0.1 (0.1)	1.7 (0.1)
	12000 ppm TCE	100 (0)	1.5 (0.2)
	100 mg/kg	0.2 (0.2)	1.8 (0.1)
	320 mg/kg	1 (0.5)	1.9 (0.2)
	1000 mg/kg	6 (4.5)	1.9 (0.1)
	1500 mg/kg	3 (0.9)	1.1 (0.2)
	2000 mg/kg	23 (6.5)	0.7 (0.3)

Table 2

1,1,1-trichlorethane (TCE) lever selection and operant response rates produced by dizocipine (MK-801), CGS-19755, L701,324 and Telazol. Numerals in brackets [] in the % TCE-lever responding column indicate number of animals in the group (n=8) that emitted sufficient responses to be included in this determination.

Drug	Drug dose or vapor concentration	% TCE-lever responding ($\pm$ SEM)	Response rate in responses/sec ($\pm$ SEM)
Dizocilpine (MK-801)	Air control	1 (0.4)	1.5 (0.2)
	12000 ppm TCE	100 (0)	1.5 (0.2)
	0.03 mg/kg	13 (12.5)	1.4 (0.2)
	0.1 mg/kg	4 (3.7)	1.9 (0.2)
	0.3 mg/kg	14	1.4 (0.2)
	0.56 mg/kg	-	0 (0)
CGS-19755	Air control	8 (9.1)	1.2 (0.2)
	12000 ppm TCE	93 (6.8)	1.4 (0.2)
	1 mg/kg	2 (0.6)	1.6 (0.2)
	3 mg/kg	1 (0.4)	1.2 (0.2)
	10 mg/kg	25 (13)	0.7 (0.1)
	17 mg/kg	-	0.2 (0.1)
L701,324	Air control	0.1 (0.1)	1.4 (0.2)
	12000 ppm TCE	99 (0.1)	1.4 (0.2)
	1 mg/kg	2 (1.8)	1.2 (0.2)
	10 mg/kg	10 (4.5)	1.2 (0.2)
	30 mg/kg	1 (0.3)	1.1 (0.2)
	56 mg/kg	2 (1.6)	1 (0.2)
Nitrous oxide	oxygen	0	1.6 (0.2)
	25% N ₂ O	6 (3.7)	1.4 (0.2)
	50% N ₂ O	15 (12.1)	1.4 (0.2)
	67% N ₂ O	3 (1.7)	0.9 (0.2)
	75% N ₂ O	7 (5.8) [7/8]	0.7 (0.2)
Telazol	Air control	3 (2.4)	1.1 (0.2)
	12000 ppm TCE	100 (0)	1 (0.1)
	3 mg/kg	3 (1.6)	1.3 (0.2)
	10 mg/kg	23 (14.7)	1.4 (0.1)
	17 mg/kg	30 (14.3)	0.7 (0.1)
	30 mg/kg	38 (18.2) [5/8]	0.5 (0.1)
	56 mg/kg	-	0 (0)