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BRIEF COMMUNICATION

Toluene Inhalation and Anxiolytic Activity: Possible Synergism with Diazepam[1]

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GELLER, I., R. J. HARTMANN, V. MENDEZ AND E. M. GAUSE. *Toluene inhalation and anxiolytic activity: Possible synergism with diazepam.* PHARMACOL BIOCHEM BEHAV 19(5) 899–903, 1983.—Toluene exposures or injections of diazepam reinstated lever responses that had been suppressed by punishment in laboratory rats. When concentrations of toluene or diazepam that were ineffective or minimally effective in this paradigm were administered in combination, they produced a qualitatively similar effect which was much greater than the sum total of effects produced by the same amount of either substance alone. These observations suggest an anxiolytic action for toluene and a possible synergism between the two substances.

Rats Toluene Diazepam Anxiolytic Conflict behavior

INHALATION of organic solvents produces euphoria, dizziness and a state of inebriation resembling that of alcohol intoxication [8]. These characteristics have led to the widespread abuse of these substances, a procedure that has been referred to as "glue sniffing" or "solvent sniffing." Toluene appears to be the principal intoxicant in many of the products that are involved in solvent sniffing [8]. An individual who sniffed toluene over a 14-year period believed the practice was no different than cigarette smoking and that toluene sniffing "relieved a state of chronic anxiety" [7]. Since little attention has been given to the potential anxiolytic activity of toluene, it was decided to evaluate the solvent in an experimental paradigm that has been demonstrated to be specific for those compounds which are clinically active as anti-anxiety drugs.

METHOD

The behavioral baseline that was used has been referred to as "experimentally-induced conflict" and has been described in detail [3]. Briefly, hungry rats are trained to press a lever for liquid food rewards that are obtainable on the average of one every 2 minutes (2-min variable interval or VI schedule of reinforcement). When the lever-pressing rates become relatively stable, clearly audible but non-aversive tone simuli of 3-minute duration are introduced at 15-minute intervals as a signal that every lever response will produce food rewards (continuous reinforcement or crf). The tone signals the change over from a relatively undesirable schedule (2-min VI) to a more desirable one with a higher "pay-off" (crf). After these contingencies are well established, conflict is induced by punishing with 60 cycle AC grid shock, any lever response made in the presence of the tone stimuli. Either high or low response rates may be obtained during the tone periods by appropriate manipulation of the shock intensities. Since drugs that are useful clinically as anti-anxiety agents attenuate conflict and increase the number of shocks a rat will take in order to obtain food rewards, a high-shock baseline was employed in this study in order to allow for a wide range of observations of possible drug effects.

Twenty male albino Sprague-Dawley rats served as subjects for this experiment. Ten of the rats had a history of twice a week exposures to 30,000 ppm toluene, during the period of 10 to 30 days of age; 10 of the rats served as fresh air controls during the same time period. At 75 days of age all rats began training on the conflict procedure. Our intent was to evaluate the rate of acquisition of conflict behavior as a function of early exposures to toluene at concentrations that have relevance to the human abuse situation.

When the rats were approximately 1 year of age, they were given a series of 10-minute exposures to high concentrations of toluene just prior to a 75-minute behavioral session on the conflict procedure. Toluene exposure concentrations of 10,000, 20,000 and 30,000 ppm were employed. Selection of these concentrations was based on the previous report that the concentration of toluene achieved when sniffing plastic cement from a paper bag is about 10,000 ppm [8]. The rats were exposed no more frequently than once a week; half of the animals received exposures in increasing order of concentrations while the other half received exposures in decreasing order of concentrations. The rats were also ex-

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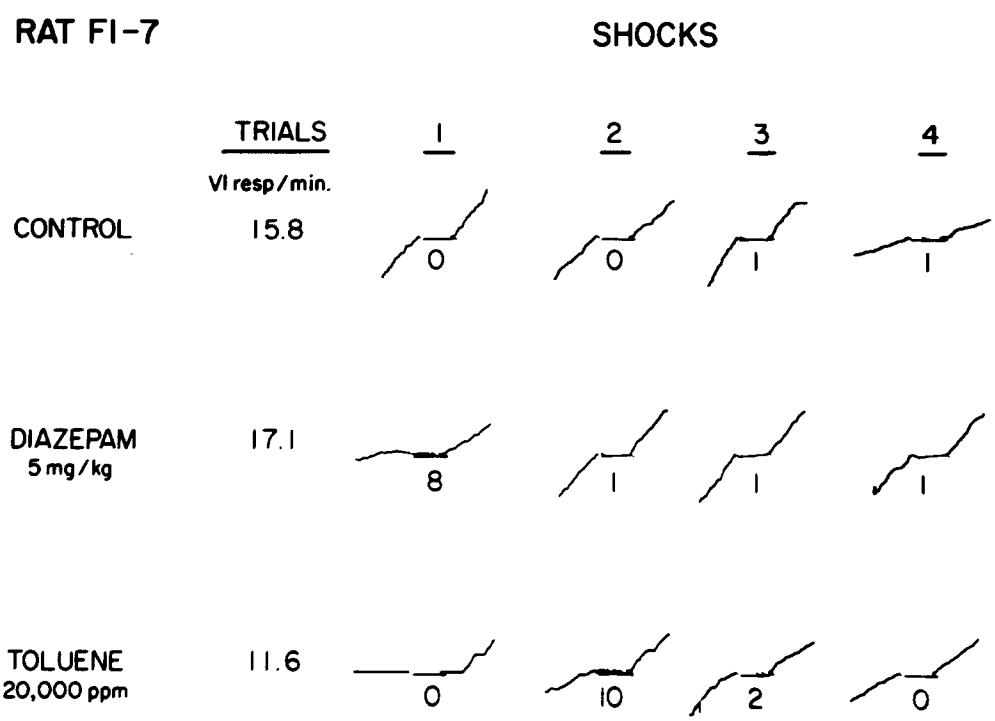

FIG. 1. Effects of diazepam and toluene on experimentally-induced conflict in the rat. Pen offsets indicate tone periods. Upward pips of the pen and numbers represent responses during the tone periods that were punished with shock and rewarded with food simultaneously.

posed to fresh air as control for 10-minute periods prior to the behavioral test session.

A large 9.125 liter desiccator fitted with a gas sampling port on the lid was used as a static exposure chamber. Toluene was introduced from a syringe onto filter paper lining the walls of the desiccator. Simultaneously with the introduction of the toluene a rat was placed in the chamber for 10 minutes. The atmosphere was sampled (0.15 ml) at 1.3-minute intervals and analyzed for toluene concentration by gas chromatography.

Since attenuation of conflict did not occur in all of the rats following exposure to toluene, only those animals for which some attenuation of conflict occurred were used for evaluating toluene-diazepam interactions. Ten rats were then given a series of intraperitoneal administrations of diazepam in doses ranging from 0.75–8.0 mg/kg. These were administered 30 minutes prior to the behavioral test session and no more frequently than once a week. Control administrations of saline were also given.

Once the diazepam conflict-attenuating doses were established, animals were administered combinations of toluene and diazepam at dose levels that were ineffective or minimally effective in attenuating conflict. Rats were administered diazepam at the individually-selected dose level 30 minutes prior to the behavioral test session and were exposed to toluene 10 minutes prior to the session. Control data were obtained by administration of saline and fresh air sham-exposure in the same time sequence.

In order to determine if the observed effects might be mediated by a diazepam-induced alteration of blood toluene, blood levels of toluene were obtained for the five rats in which the greatest amount of diazepam-toluene effects were evident. The rats were treated with toluene alone or toluene and diazepam in the exact concentrations previously shown to attenuate conflict behavior when the substances were administered in combination. Tail-vein blood samples of 0.2 ml were obtained immediately following exposure and every 15 minutes thereafter during a 75-minute period, so that blood sampling would correspond with the occurrence of conflict trials. Concentrations of toluene in blood were determined by gas chromatography using a modification of the Wallace and Dahl headspace technique for blood alcohol [9].

RESULTS

Figure 1 contains data of rat FI-7 taken from 75-minute behavioral sessions under control, diazepam and toluene conditions. The top row of cumulative records shows the degree of conflict attenuation (number of shocks taken) under control conditions. The criterion for acquisition of the conflict behavior was reached when each animal took no more than 3 shocks during a 4-trial behavioral test session. Intergroup comparisons of the mean number of trials±S.E.M. for those animals exposed during 10–30 days of age with the data of the sham-air controls yielded values of 40.89±1.05 and 40.22±0.36 respectively. A comparison of the mean shock voltages±S.E.M., when the acquisition criterion was reached by all rats, was 2.95±0.25 for the exposed rats and 2.73±0.23 for the sham-exposed animals. Acquisition of conflict behavior was not significantly affected as a function of early exposure to high concentrations of toluene.

The bottom row of cumulative records shows the attenuation of conflict resulting from a 10-minute exposure to 20,000 ppm toluene. The rat tolerated 12 shocks as compared with only 2 shocks under control conditions. This effect has been demonstrated previously [5] to be characteristic of clin-

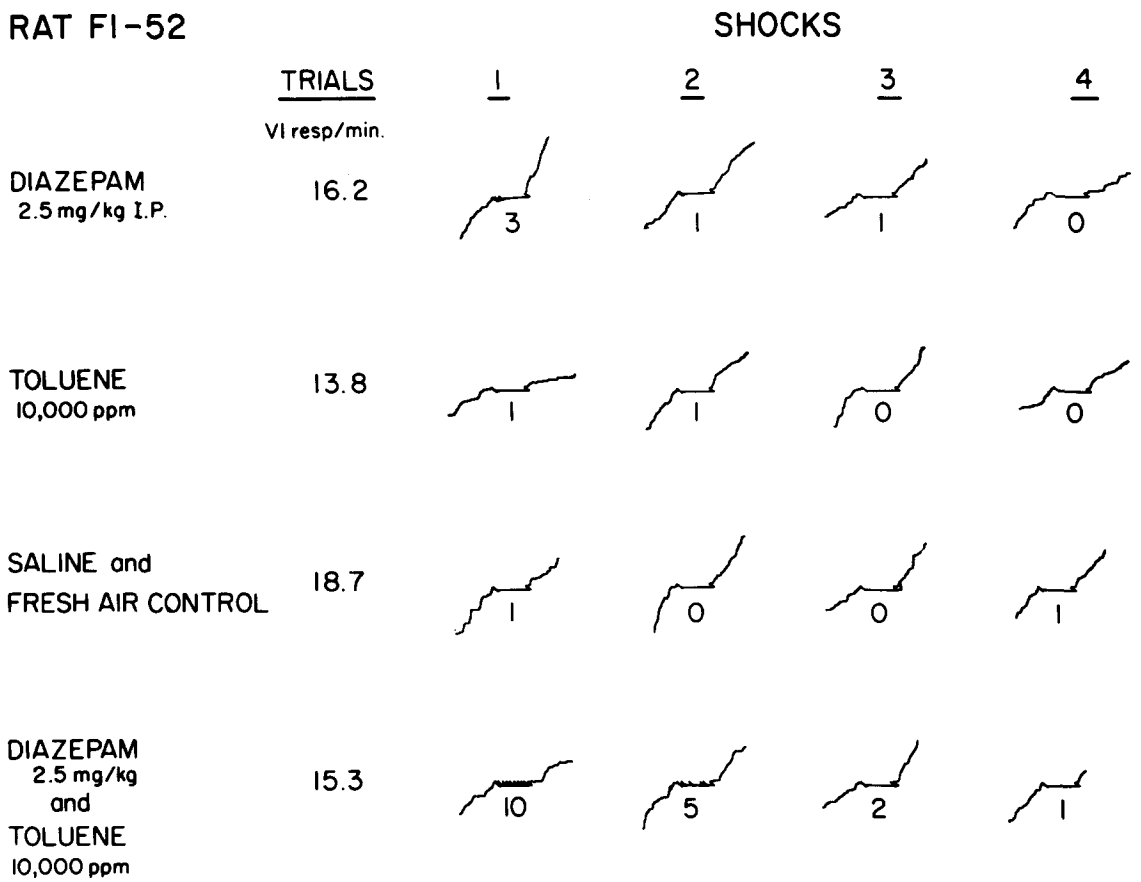

FIG. 2. Effects of toluene and diazepam administered alone and in combination on experimentally-induced conflict in the rat. Pen offsets indicate tone periods. Upward pips of the pen and numbers represent responses during the tone periods that were punished with shock and rewarded with food simultaneously.

ically active anxiolytics such as chlordiazepoxide, oxazepam and diazepam. The variable interval response rate decreased from 15.8/min during the control session to 11.6/min following exposure to toluene. The reduction in variable interval response rates, which was greatest during exposures to 30,000 ppm toluene, was probably due to the sedative action of toluene and, as has been shown with anxiolytics [4], it tended to mask the anti-conflict effects during the tone periods.

Exposure to toluene produced qualitatively similar effects in 10 of the 20 animals. Six of the rats were from the previously exposed group and 4 were from the sham-exposed group. Therefore it appears that the toluene-induced attenuation of conflict is not related to early toluene exposure.

The middle row of cumulative records shows the attenuation of conflict produced by 5.0 mg/kg diazepam. The rat tolerated 8 shocks during the first trial and 1 shock on each succeeding trial of the session. The variable interval response rate increased to 17.1 resp/min from 15.8 during the control session.

Figure 2 illustrates the effect on conflict resulting from combined administration of concentrations of diazepam and toluene that were ineffective when administered alone. The variable interval response rates decreased below the control value of 18.7/min under all treatment conditions. Under 2.5 mg/kg diazepam the rat took 5 shocks during 4 conflict trials. After exposure to 10,000 ppm toluene the rat took only 2 shocks just as during the control session. However, when the animal was exposed to 10,000 ppm toluene following administration of 2.5 mg/kg diazepam, the rat took a total of 18 shocks during the 4 trials.

Similar data for all 10 subjects are shown in Fig. 3. The control bars represent an average of the saline control, the fresh air control and the saline plus fresh air control. In 7 of the 10 subjects the effects resulting from combining diazepam and toluene are considerably greater than the effects produced by either substance alone and are greater than the sum of the individual effects. This is best illustrated by the data of rat 67 who took 2 shocks under 2.0 mg/kg diazepam, 1 shock under 5,000 ppm toluene and 22 shocks under the combination, an effect 7 times greater than the sum of the individual effects of either substance. In an assessment of statistical significance, these data were subjected to a repeated measures analysis [6]. The results indicate a significant treatment effect, $F=15.79$, $p<0.01$, $df=3,27$.

The blood concentrations of toluene after 15 minutes recovery time and thereafter were generally quite similar under both treatment conditions. However, immediately after exposure to toluene the blood levels of toluene were considerably lower under diazepam and toluene than under toluene alone. This was true for 4 of the 5 test animals; decreases in blood toluene concentrations of from 30 to 70% of that resulting from exposure to toluene alone were observed after exposure to toluene plus diazepam.

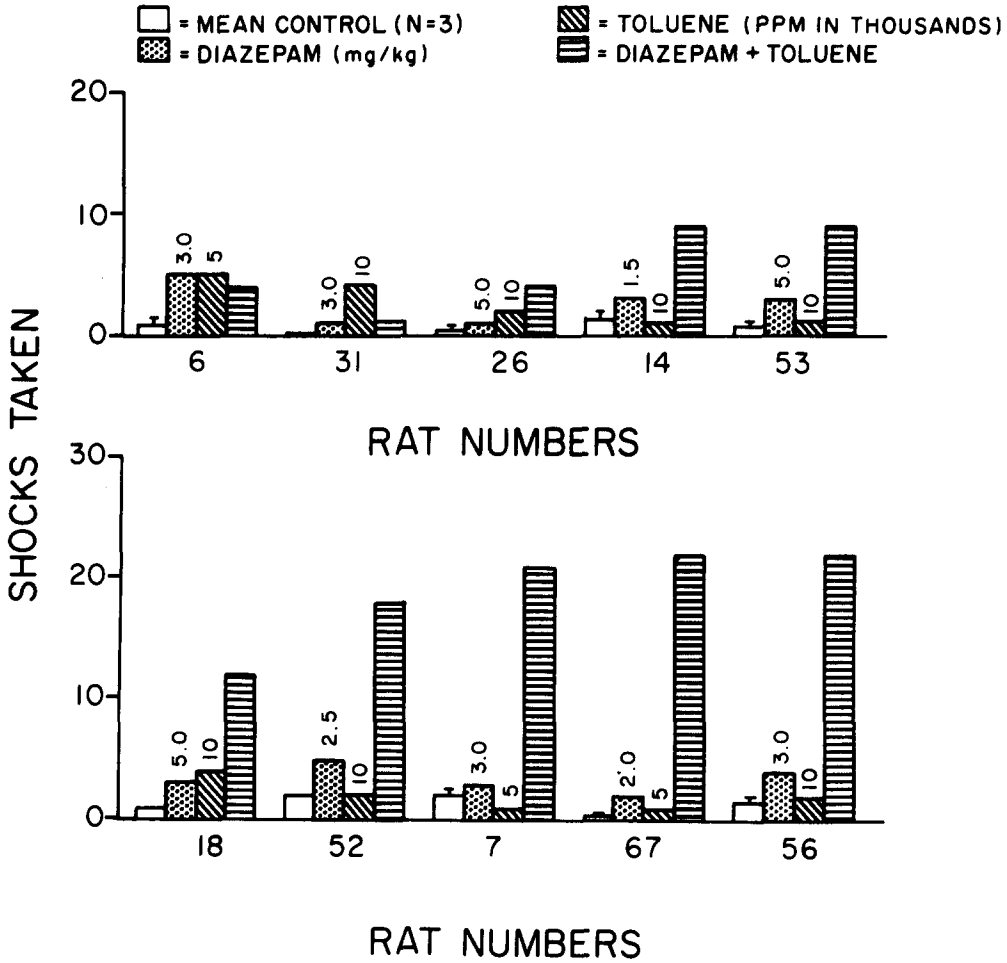

FIG. 3. Effects of toluene and diazepam administered alone and in combination on experimentally induced conflict in the rat. Control data were derived from averages of saline alone, fresh air control and saline in combination with fresh air control.

DISCUSSION

The findings of this investigation demonstrate that toluene, a major component of many substances that are abused via inhalation, produces behavioral activity in animals qualitatively similar to that produced by clinically active anti-anxiety drugs. The potential anxiolytic activity of toluene is not wholly unexpected in view of a previous report that sniffers were extremely high in anxiety as measured by the Taylor Manifest Anxiety Scale [1,2].

Other investigators have also reported that toluene and xylene produce effects in animals qualitatively similar to those produced by minor tranquilizers. Wood *et al.* [10] reported that like minor tranquilizers, toluene or xylene delayed pentylenetetrazol induced convulsions while toluene inhalation delayed the time to death after injection of pentylenetetrazol as a function of duration and concentration of toluene exposure. They also reported that like minor tranquilizers, toluene attenuated the suppression of operant responding that had been produced with electric shock punishment of rats working on a multiple schedule of reinforcement.

Combinations of ineffective concentrations of toluene administered in combination with ineffective concentrations of the anxiolytic, diazepam, produced anxiolytic effects that probably are indicative of a large synergistic action between the two substances. This observation could in part account for polydrug abuse among "glue sniffers." Since diazepam, other benzodiazepines and certain classes of barbiturates are also heavily abused drugs, it is possible that abuse liability may be linked to their anxiolytic or sedative action or an interaction between the two. In addition, as far as we are aware, although the literature is replete with chemical classes studied in receptor binding, there is no evidence to indicate whether toluene has or has not any displacement effects on benzodiazepine-GABA binding systems. It would be quite important to determine whether the suggested anxiolytic properties of toluene are mediated via the benzodiazepine-GABA receptor complex.

Following administration of diazepam in combination with exposure to toluene, decreases in blood toluene concentration were observed in the amount of 30 to 70% of that resulting from exposure to toluene alone; yet a significant potentiation of effect was produced by the combination. This potentiation could possibly be due to competition between toluene and diazepam in plasma for hydrophobic binding sites on blood transport proteins; displacement of bound toluene by diazepam could result in an increase in the amount

of free, dissolved toluene crossing the blood-brain barrier. However, this observation alone does not explain the mechanism of potentiation seen here. These experiments did not examine either: (1) possible dose-effects of diazepam upon respiration or volume of toluene inhaled; or (2) interactive effects of toluene upon diazepam metabolism or diazepam upon toluene metabolism. These avenues of study have become of interest in light of the present findings.

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