Propylene Glycol Elicits Anxiolytic-like Responses to the Elevated Plus-maze in Male Mice

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Abstract

Propylene glycol is a common solvent often contained in injectable solutions of anxiolytics of low water-solubility, such as diazepam (Valium) and pentobarbital (Nembutal). Several studies have shown that propylene glycol can have an inhibitory effect on the central nervous system. This study, using ethanol for comparison, further examined whether propylene glycol has anti-anxiety properties.

Use of the elevated plus-maze test with male mice revealed that propylene glycol at doses (27 or 41 mmol kg^{-1}, i.p.) which did not affect general activity, increased the number of entries into open arms and of head dips over open arm edges, indicative of an anxiolytic effect. In parallel, ethanol (14 and 27 mmol kg^{-1}, i.p.) caused an increase in the amount of time spent on open arms and number of entries into open arms, accompanied by reduction of returns into closed arms. These doses of ethanol had no significant effect on motor ability.

The results suggest that propylene glycol can act as an anxiolytic agent and that its anxiolytic potency is weaker than that of ethanol. In addition to previous warnings about the pharmacological effects of propylene glycol, the findings of this study alert investigators to the anxiolytic properties of the compound when it is employed as a solvent in anxiety or anxiety-related studies.

Propylene glycol (propane-1,2-diol) is a dihydric alcohol commonly used as a vehicle for hydrophobic drugs, as a stabilizer in vitamin preparations and as a preservative in parenteral, oral and topical formulations. Although the compound was originally thought to be an inert glycol, accumulated data have suggested a variety of pharmacological activity, including reduction of methoxamine-induced bronchoconstriction, depression of cardiovascular function, and protection against ethanol-induced lethality. In particular, data derived from different laboratories have suggested an inhibitory effect on the central nervous system. Injection of the glycol resulted in reduced locomotor activity, body and limb tone and respiration rate, and suppression of secondary conditioned responses in mice and rats (Singh et al 1982). Pretreatment with the glycol delayed the onset of seizure caused by exposure to hyperbaric oxygen in cats (Beckman & Crittenden 1981). Propylene glycol enhances the hypnotic effect of pentobarbital in rodents (Singh et al 1982) and the sedative effect of diazepam in man (Forrest & Galletly 1988).

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A drug vehicle should be inactive, i.e. without pharmacological influence on the effects of the primary drug. Propylene glycol as a solvent or co-solvent is often included in solutions of psychoactive drugs of low water-solubility, e.g. diazepam (Valium, Roche) and pentobarbital (Nembutal, Abbott). The findings described above imply that the glycol contributes to the effects of these drug solutions both physicochemically and pharmacologically. Because anxiolysis is an important action of diazepam and pentobarbital, it is of particular interest to know whether propylene glycol is active in this respect. Moreover, because propylene glycol and ethanol are common organic solvents, high caloric substances, and depressants of the central nervous system, many experiments have been performed to compare their properties (Newman & Lehman 1936–1937; Hanzlik et al 1939; Latven & Molitor 1939). Although ethanol has been shown to have anti-anxiety properties (Prunell et al 1994), there has been no comparison of the capacity of the two alcohols to regulate anxiety.

The main purpose of this study was to examine the effect of propylene glycol on anxiety and to compare the anxiolytic action of the glycol and

ethanol. The elevated plus-maze test was employed as an animal model of anxiety. Conventionally, a set of spatial and temporal measures is used to index the level of anxiety and general activity on the plus-maze (Lister 1987). In recent years, several other ethological measures have been developed to improve the sensitivity of the model and the reliability of measurements obtained from its use (Cole & Rodgers 1994). In this study, both traditional and novel measures were adopted to monitor drug effects.

Materials and Methods

Drugs

(±)-Propylene glycol (BDH, Australia) and ethanol (CSR, Australia) were dissolved in 0·9% NaCl (saline). All injections were given intraperitoneally, in a volume of 10 mL kg^{-1}, 20 min before behavioural testing. Selection of the doses of propylene glycol (14, 27 or 41 mmol kg^{-1}) was based on the consideration that 14 mmol kg^{-1} ($\sim$10% in 10 mL kg^{-1}) was ineffective in behavioural tests reported elsewhere (Singh et al 1982) and that 61 mmol kg^{-1} ($\sim$45% in 10 mL kg^{-1}) resulted in significant impairment of motor ability on the plus-maze (Lin et al unpublished data). The doses of ethanol (14 and 27 mmol kg^{-1}) were chosen to match those of propylene glycol.

Animals

Subjects were experimentally naive, male Quackenbush Swiss mice (University of Sydney Laboratory Animal Service, Australia), 11–14 weeks. They were housed in groups of ten with free access to water and standard laboratory chow. The colony was maintained at 21±1°C and on a 12-h light–dark cycle.

Apparatus and procedures

The plus-maze comprised two pairs of opposing open and closed arms (30 cm × 5 cm) which extended from a central area (5 cm × 5 cm). The maze floor, constructed from black Plexiglas, was 40 cm above the room floor. The open arms included a clear, slightly raised edge (0·25 cm height) to reduce the likelihood of animals falling-over (Cole & Rodgers 1994). The closed arms were enclosed with three roof-open walls (15 cm height) made from clear Plexiglas (Lister 1987; Cole & Rodgers 1994). The maze was located at the centre of an area enclosed by a white, four-sided fence (180 cm × 120 cm for each side). The testing room was illuminated by four white, 40-W fluorescent tubes hung at the ceiling level. A video camera was

mounted vertically approximately 1·5 m above the plus-maze for recording behavioural responses.

Mice were randomly allocated to experimental groups and then adjusted slightly to match the average body weights of the groups. All behavioural tests were conducted between 1030 and 1630 h, and the testing order for different treatments was counterbalanced. For habituation animals were brought into an injecting room approximately 1 h before behavioural testing. Immediately after injection the mice were separately detained in small boxes (29 cm × 16 cm × 11 cm) and then tested individually in a testing room. At the beginning of a test each mouse was placed on to the central area of the maze facing an open arm. Its behaviour on the plus-maze was recorded for 5 min. After each run the mouse was returned to its home cage and the maze was cleaned with a wet sponge. The tape-recorded behaviour was scored by a trained observer who was blind to the treatment conditions.

The traditional measures comprise the amount of time (%) spent on open arms (denoted % open time), entries into open arms (% open entries), and the number of entries into closed arms (closed entries). Arm entry was defined as all four paws having crossed the dividing line between an arm and the central area. The novel ethological measures were head dip over the edges of open arms (denoted head dip; defined as head and shoulder moving beyond the edges of open arms and down towards the floor); closed arm return (closed return; defined as moving towards the open end of a closed arm and then returning towards its closed end without visiting other arms); and stretch-attend posture (stretch posture; defined as stretching forward and then retracting to the original position without any forward locomotion).

Statistics

Raw data for the propylene glycol experiment were subjected to an unpaired t-test. Raw data for the ethanol experiment were analysed by one-way analysis of variance followed by Fisher's PLSD test where appropriate. Significance levels were set at $P < 0.05$.

Results

The effects of propylene glycol on the results from the plus-maze test are presented in Table 1. Using the conventional measures the compound at the highest dose significantly ($P < 0.05$) increased the number of open entries but had no effect on open time or closed entries. Using the ethological measures the two higher doses elicited a statistically

Table 1. The effect of propylene glycol on responses to the elevated plus-maze in male mice.

Dose (mmol kg^{-1})	n	Open time (%)	Open entries (%)	Closed entries	Head dips	Closed returns	Stretch posture
0	13	41·1±4·5	42·9±3·7	10·2±0·6	14·2±2·0	2·4±0·5	6·4±1·5
14	13	42·4±5·4	43·4±4·5	10·8±0·6	17·1±3·3	1·5±0·5	5·0±1·3
0	15	39·7±2·9	38·5±2·8	9·9±0·5	10·9±1·0	1·5±0·3	5·9±0·9
27	15	42·0±4·4	45·1±4·1	10·0±0·7	25·3±3·1†	2·2±0·4	4·2±0·8
0	15	28·7±4·0	28·7±3·3	10·6±0·8	10·9±1·8	2·3±0·6	6·5±1·2
41	15	33·2±4·8	40·2±3·9*	9·9±1·2	22·9±3·5†	2·2±0·5	4·2±0·8

Propylene glycol or normal saline was administered intraperitoneally 20 min before a 5-min test on the elevated plus maze. Data are expressed as means±s.e.m. *$P < 0.05$, †$P < 0.01$, significantly different from result for control group (unpaired t-test).

Table 2. The effect of ethanol on responses to the elevated plus-maze in male mice.

Dose (mmol kg^{-1})	n	Open time (%)	Open entries (%)	Closed entries	Head dips	Closed returns	Stretch posture
0	8	19·0±6·5	25·5±7·7	13·3±1·5	7·0±2·9	4·3±1·0	4·9±1·3
14	9	43·5±7·8*	46·4±6·9*	12·7±1·3	16·3±4·8	0·9±0·5†	3·2±1·0
27	9	50·3±6·5†	53·9±5·2†	13·4±1·3	22·1±5·3	1·7±0·8*	1·7±0·9

Ethanol or normal saline was administered intraperitoneally 20 min before a 5-min test on the elevated plus-maze. Data are expressed as means±s.e.m. *$P < 0.05$, †$P < 0.01$, significantly different from result for control group (one-way analysis of variance followed by Fisher's PLSD test).

reliable (for both $P < 0.01$) increase of head dips without significant effect on closed returns and stretch postures.

The effects of ethanol are depicted in Table 2. It can be seen from the conventional measures that both doses of the drug elicited a marked increase in open time and open entries ($P < 0.05$ or better) but had no significant effect on closed entries. The ethological measures showed that both doses elicited a significant reduction in closed returns ($P < 0.05$ or better) and, in parallel, induced an increasing trend in head dips and a decreasing trend in stretch postures although these effects failed to reach a significant level.

Discussion

The main purpose of this study was to test the effects of propylene glycol on anxiety. Results from the elevated plus-maze test demonstrate that propylene glycol, at dose levels which did not change an animal's general activity on the maze, dose-dependently increased the number of open entries and head dips. The data provide novel evidence for the anxiolytic properties of this common solvent. The results also show that ethanol increased the amount of open time and the number of open entries and reduced closed returns, in agreement with many previous studies (e.g. Prunell et al 1994) and confirming that the experimental settings in the current study were appropriate.

This study is the first to demonstrate the anti-anxiety effect of propylene glycol on results from the elevated plus-maze. Chenoweth & Hendershot (1965) reported that after oral administration of S-propylene glycol a caged wild rat became less anxious when handled. The method used by Chenoweth & Hendershot was one of general behavioural observation and was not specific for the study of anxiety. In the current study their finding was extended on the basis of the data derived from a well-validated animal model of anxiety, the elevated plus-maze test (Lister 1987; Cole & Rodgers 1994). Brown (1968), using an avoidance-behaviour paradigm, failed to observe an anxiolytic effect of 65·7 mmol kg^{-1} (5 g kg^{-1}) racemic propylene glycol in male weanling Sprague-Dawley rats. Whether the differences in subjects, animal models and drug doses between Brown's and our experiments account for the discrepancy remains to be clarified.

Previous pharmacological studies have shown that both propylene glycol and ethanol exert inhibitory effects on the central nervous system, and the effects of propylene glycol are generally weaker than those of ethanol. For example, the narcosis (Newman & Lehman 1936–1937) and hypnotic effect (Latven & Molitor 1939) induced by propylene glycol were only one-third and one-fifth, respectively, of those induced by ethanol and the anaesthetic dose of propylene glycol was about six times that of ethanol (Hanzlik et al 1939). A similar potency relationship between the two drugs'

anxiolytic effects was seen in the current study. At a level of $14\,mmol\,kg^{-1}$, ethanol induced a significant anxiolytic effect across three behavioural measures in sharp contrast with the ineffectiveness of propylene glycol. At a higher dose $(27\,mmol\,kg^{-1})$ ethanol continued to be effective on the same three measures whereas the effect of propylene glycol was only significant for one measure. The group sizes in the propylene glycol experiment $(n = 13-15)$ were larger than those in the ethanol experiment $(n = 8-9)$. These results indicate that although propylene glycol can work as an anxiolytic agent its effect is weaker than that of ethanol.

The behavioural effects of propylene glycol suggest that caution should be exercised when the compound is used as a solvent in solutions of psychoactive drugs. Warnings about this problem have previously been made by several laboratories (e.g. Peeters et al 1992). In particular, Singh et al (1982) recommended 10% $(14\,mmol\,kg^{-1}$ in $10\,mL\,kg^{-1})$ as an upper safety limit for the use of the glycol as a solvent, because no significant behavioural effect was observed at this dose level in their experiments with rats and mice. This is in accord with the effects of propylene glycol on the plus-maze results in the current study. Employment of high concentrations of propylene glycol as a solvent (e.g. 40% in $10\,mL\,kg^{-1}$ $(55\,mmol\,kg^{-1})$ Biscoe & Fry (1982); and 100% in $5\,mL\,kg^{-1} \times 2$ $(137\,mmol\,kg^{-1})$ Tuo et al (1996) in mice and $5\,g\,kg^{-1}$ $(65.7\,mmol\,kg^{-1})$ Brown (1968) in rats) is occasionally seen in the literature. It is worth noting that even at doses as low as $0.1-0.2\,mL\,kg^{-1}$ $(1.4-2.7\,mmol\,kg^{-1})$ propylene glycol delayed the onset of oxygen-induced convulsion in cats (Beckman & Crittenden 1981). This indicates that the minimum effective dose of the solvent depends upon the animal model used.

Although the behavioural effects of propylene glycol have been repeatedly reported, the neural mechanisms underlying these effects have not been clearly elucidated. Beckman & Crittenden (1981) suggested GABAergic involvement in the anticonvulsion effect of propylene glycol. Given that the role of $GABA_A$ receptors in mediation of the anxiolytic effects of benzodiazepines, barbiturates and ethanol has been well established (Hobbs et al 1996), it seems reasonable to propose a GABAergic mechanism for the effects of propylene glycol on the results from the plus-maze test. Nevertheless, in a $[^3H]$muscimol binding assay using rat cerebral cortical synaptosomes as a $GABA_A$ receptor preparation, we found that the compound only weakly inhibited $[^3H]$muscimol binding to $GABA_A$ receptors (IC50 > 2 mM; Lin et al unpublished data),

indicating that propylene glycol might have no direct effects on postsynaptic $GABA_A$ receptors.

Propylene glycol is a racemic mixture. There are indications in the literature of differences between the pharmacological action of the two enantiomers. Brown (1968) observed that R-propylene glycol is uniformly more potent than the S enantiomer in enhancing hexobarbital sleeping time, in protecting against electrically-induced convulsion and in reducing avoidance behaviour in laboratory rats and mice. These differences in potency were ascribed to the higher rate of clearance of the S enantiomer. In contrast, Chenoweth & Hendershot (1965) reported that S-propylene glycol had a stronger ataractic effect than the racemic compound in wild rats, the R enantiomer having almost no effect. Further investigations are warranted to clarify the pharmacological properties of these compounds.

In summary, this study demonstrates that propylene glycol has anti-anxiety properties and that its anxiolytic effects on results from the plus-maze test are weaker than those induced by ethanol. The results should alert investigators to the intrinsic pharmacological action of the glycol when it is employed as a solvent in psychopharmacological studies.

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