

Interference of propylene glycol with the hole-board test

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Abstract

Experimental drugs and/or plant extracts are often dissolved in solvents, including propylene glycol. Nevertheless, there is evidence for psychoactive properties of this alcohol. In this study we found that in the hole-board test 10% propylene glycol did not modify the head-dipping behavior. However, 30% propylene glycol induced an increase in the number of head-dips (46.92 ± 2.37 compared to 33.83 ± 4.39 , $P < 0.05$, ANOVA/Student-Newman-Keuls), an effect comparable to that obtained with 0.5 mg/kg diazepam (from 33.83 ± 4.39 to 54 ± 3.8 , $P < 0.01$, ANOVA/Student-Newman-Keuls). These results demonstrate that 30% propylene glycol has significant anxiolytic effects in this model and therefore cannot be used as an innocuous solvent.

Key words

- Screening
- Anxiolytic drugs
- Propylene glycol
- Diazepam
- Solvents
- Hole-board

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Solvents are frequently used as vehicles for experimental drugs and/or plant extracts that cannot be dissolved in water or saline. Among solvents, propylene glycol (PPG) is fairly commonly used in experimental pharmacology and toxicology. PPG is also often used as excipient in injectable formulations including anxiolytics (e.g., in Brazil, Diazepam N.Q.[®] (Novaquímica), Contaz (Cristália), Diazepam (Eurofarma), Diazepam (União Química), Diazepam (Vital Brazil), and Somaplus (Cazi)).

Indications that PPG is active in the central nervous system have been reported. PPG (*ip*) at concentrations of 50 to 100% produced a moderate to marked decrease in spontaneous motor activity, body and limb tone, and respiration; moreover, 60% of the animals showed ptosis and ataxia. However, these effects were found to be negligible at

lower concentrations (10 to 20%) (1). While clonazepam dissolved in PPG did not significantly protect cats from high-pressure oxygen-induced convulsions, PPG alone at doses of 0.1-0.2 ml/kg (*po*) did offer significant protection (2). An increase in the number of open arm entries and head-dips in male mice that received PPG at doses of 27 to 41 mmol/kg (*po*) (approximately 20 to 30%) in the elevated plus-maze test has been recently reported (3). These data provide evidence for the anxiolytic properties of this alcohol.

The hole-board has gained popularity as a model of anxiety, offering "a simple method for measuring the response of an animal to an unfamiliar environment, with advantages that several behaviors can be readily observed and quantified in this test" (4). The purpose of the present paper is to report the effects of PPG in the hole-board model, as a cautionary

note about the use of PPG as solvent in this model.

Experiments were performed with male adult (CF1) mice received from Fundação Estadual de Experimentação e Produção da Saúde immediately after weaning (21 days). Animals were maintained in our own animal facilities in a controlled environment ($22 \pm 1^\circ\text{C}$, 12-h light/dark cycle, free access to food (Nuvilab CR1) and water) up to 10 weeks old (25-40 g). Diazepam and PPG were acquired from Sigma Chemical Co., St. Louis, MO, USA. Vehicle concentration is reported as percent in 0.9% NaCl (saline). Diazepam at a concentration of 0.5 mg/kg was suspended in 10% (v/v) or 30% (v/v) PPG.

The hole-board apparatus (Ugo Basile, Varese, Italy) consisted of gray Perspex panels (40 x 40 cm, 2.2 cm thick) with 16 equidistant holes 3 cm in diameter in the floor. Photocells below the surface of the holes measured the number of head-dips. The board was positioned 15 cm above the table and divided into 9 squares of 10 x 10 cm with a water-resistant marker. Mice were

transported to the dimly lit laboratory at least 1 h prior to testing. Treatments (saline, 10% PPG, 30% PPG, 0.5 mg/kg diazepam solubilized in 10 or 30% PPG) were administered *ip*, 10 ml/kg, 30 min prior to testing ($N = 10-12/\text{group}$). Each animal was placed singly in the center of the board facing away from the observer and its behavior recorded for 5 min; the latency to the first head-dip was measured using a stopwatch (5). Rearing, grooming, number of defecations and spontaneous movements (number of squares crossed with all four paws) were also recorded. Results are reported as means \pm SEM. The numbers of head-dips, rearing, grooming, spontaneous movements and latency were analyzed by ANOVA followed by the Student-Newman-Keuls test.

Head-dip results are shown in Figure 1. Diazepam (0.5 mg/kg) dissolved in 10 or 30% PPG significantly increased the number of head-dips in comparison to saline or 10% PPG ($P < 0.01$). PPG (10%) did not modify the head-dipping behavior. However, 30% PPG alone induced an increase in head-dips in comparison with saline ($P < 0.05$), i.e., an increase comparable to that induced by diazepam. Data about spontaneous locomotion (number of squares crossed with all four paws) are presented in Figure 2. Regardless of the PPG concentration used in the solution, locomotion increased in animals treated with diazepam (0.5 mg/kg) in comparison with saline, 10% PPG or 30% PPG. PPG alone did not influence locomotion. Neither diazepam nor PPG significantly altered the number of rearing, grooming, or defecation events or latency to first head-dip (data not shown).

Figure 1 - Effects of diazepam (DZP) and propylene glycol (PPG) on the number of head-dips in the hole-board test. PPG (10%, PPG10), 30% PPG (PPG30), and 0.5 mg/kg diazepam diluted in 10% PPG (DZP10) or 30% PPG (DZP30) were used. Each column represents the mean \pm SEM. * $P < 0.05$ and ** $P < 0.01$ compared to saline (ANOVA).

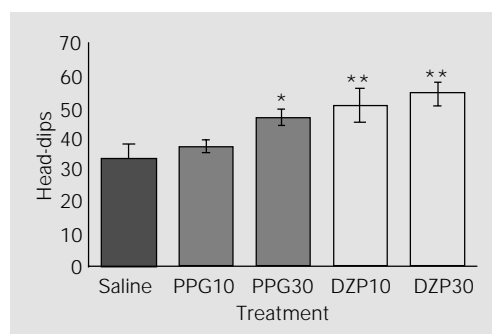
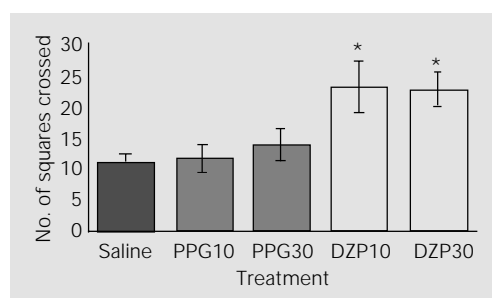


Figure 2 - Effects of diazepam (DZP) and propylene glycol (PPG) on locomotion (number of squares crossed with all four paws) in the hole-board test. PPG (10%, PPG10), 30% PPG (PPG30), 0.5 mg/kg diazepam diluted in 10% PPG (DZP10) or 30% PPG (DZP30) were used. Each column represents the mean \pm SEM. * $P < 0.05$ compared to saline (ANOVA).



studies have reported that diazepam increases locomotor activity in the open field (6-8). Moreover, increases in number of squares crossed in the open field and in head-dips in the hole-board were obtained with desmethyldiazepam and chlordesmethyldiazepam (9). Our results indicate that this non-sedative but anxiolytic dose of diazepam facilitates exploratory behavior expressed as increased head-dips and locomotion.

The significant new finding of this study is that 30% PPG alone increases head-dip behavior. PPG, 10 or 30%, did not affect any of the other parameters observed. Although toxicity studies showed that toxic PPG doses

are much higher than those used in this study (10,11), our results showed that 30% PPG can interfere with results obtained with the hole-board model. The data are in agreement with previous results (3) indicating that 10% is the limiting concentration of PPG as an inert solvent in the elevated plus-maze test.

The data reported here are of relevance in selecting vehicles for screening candidate compounds for new sedative drugs (e.g., 12), since they indicate that PPG does interfere with the hole-board model. The data also indicate that caution is necessary when using PPG as excipient in pharmaceutical products.

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