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The effect of solvents on the potency of chlordiazepoxide, diazepam, medazepam and nitrazepam

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ED50 values for loss of righting reflex in mice have been determined for a series of benzodiazepines after intraperitoneal injection of the drugs in various vehicles. The vehicles used greatly modified the ED50 values obtained. The effects obtained were due either to a failure of the vehicle to achieve or maintain complete solubilization of the drug, or to the pharmacological action of the vehicle modifying that of the drug. Diazepam, medazepam and nitrazepam are insoluble in water, but are soluble in dimethylsulphoxide (DMSO) and in aqueous vehicles containing glycofurol or propylene glycol. Chlordiazepoxide hydrochloride is water-soluble. Similar ED50 values were obtained in experiments where the benzodiazepines were injected in an aqueous carboxymethylcellulose suspension and in experiments where the drugs were dissolved in DMSO. Lower ED50 values were obtained when the drugs were dissolved in vehicles containing propylene glycol or glycofurol. The increase in potency of the benzodiazepines could be ascribed either to the solubilization of the drugs or to the synergistic pharmacological activity of the solvents. An ED50 value for diazepam, which was not influenced by the pharmacological activity of the solvent, could be obtained using the vehicles containing either glycofurol or propylene glycol. For medazepam and nitrazepam, the solvent mixtures containing propylene glycol and glycofurol respectively were required to avoid drug-solvent interactions.

In clinical practice the benzodiazepines are administered parenterally for the therapy of epileptiform seizures (Gastaut, Naquet & others, 1965), muscle spasm and tetanus (Weinberg, 1964) and for premedication and the induction of anaesthesia (see Knight & Burgess, 1968, for references).

Chlordiazepoxide is manufactured as the hydrochloride salt which is freely soluble but unstable in aqueous solution (Randall, 1961). As the benzodiazepines, diazepam, nitrazepam and medazepam, are insoluble in water, other solvents are used in formulations of these drugs for injection. Formulations containing glycofurol (Spiegelberg, Schläpfer & others, 1956) and propylene glycol have been used commercially as vehicles for diazepam.

Preliminary screening of glycofurol and propylene glycol has shown that, like the benzodiazepines, they may produce hypnosis, motor inco-ordination, depression of polysynaptic reflexes and ataxia (unpublished observations). In the present experiments the interactions of various solvents with the benzodiazepines, chlordiazepoxide HCl, diazepam, medazepam (Ro5-4556) and nitrazepam have been examined using loss of the righting reflex in mice as a measure of pharmacological activity.

METHODS

The benzodiazepines, chlordiazepoxide HCl (Librium), diazepam (Valium), medazepam (Nobrium) and nitrazepam (Mogadon), were formulated in one of the following vehicles for intraperitoneal injection:

(a) Dimethylsulphoxide. (b) Propylene glycol (45% v/v), ethanol (10% v/v), benzyl alcohol (1·5% v/v), sodium benzoate (9·8% w/v), benzoic acid (0·24% w/v) in water. (c) Glycofurol (45% v/v), ethanol (10% v/v), benzyl alcohol (1·5% v/v) in water. (d) A suspending vehicle of sodium carboxymethylcellulose (0·5% w/v), Polysorbate 80 (0·5% v/v), phenylmercuric nitrate (0·001% v/v) in water. In the text these formulations are referred to as DMSO, propylene glycol solvent, glycofurol solvent and the methylcellulose vehicle respectively. Vehicles (b) and (c) have been used as solvents for diazepam in a commercial preparation for parenteral use (Valium ampoules, Roche).

Each of the benzodiazepines is soluble in formulations (a), (b) and (c) at a strength of 5 mg/ml of vehicle. Solutions using these vehicles were injected intraperitoneally, without dilution, using a micro-syringe. Drugs in formulation (d) were injected after suitable dilution using a 1·0 ml tuberculin syringe.

White Swiss mice (Commonwealth Serum Laboratories), of either sex, 20–30 g, were weighed individually and the various formulations administered intraperitoneally. Animals were tested for loss of righting reflex for 3 min during a test period of 13 min from the time of injection. ED50 values were calculated by the method of Litchfield & Wilcoxon (1949), using groups of 10 or 20 mice at each dose. Drug formulations were prepared immediately before each experiment.

RESULTS

Table 1 shows the ED50 values for chlordiazepoxide HCl, diazepam, nitrazepam and medazepam (at 5 mg/ml) in the different vehicles. The relative potencies of the benzodiazepines are also shown (chlordiazepoxide HCl = 1). The ED50 values and the relative potencies of the benzodiazepines are similar in the methylcellulose vehicle and in DMSO but lower values were obtained with the other vehicles.

Table 1. *ED50 values (mg/kg) for benzodiazepines (5 mg/ml) in various formulations.*

	Methylcellulose vehicle		DMSO		Propylene glycol solvent		Glycofurol solvent	
	ED50	RP	ED50	RP	ED50	RP	ED50	RP
Chlordiazepoxide HCl	35·4 (37·2–33·5)	1·0	34·8 (40·0–30·3)	1·0	30·8 (37·0–25·7)	1·0	14·1 (17·6–11·3)	1·0
Diazepam	25·6 (33·2–19·7)	1·4	18·5 (23·5–14·8)	1·9	2·8 (3·3–2·0)	11·0	2·9 (3·5–2·4)	4·8
Medazepam	42·2 (51·3–33·1)	0·8	31·5 (34·6–28·6)	1·1	16·5 (17·8–15·3)	1·9	10·5 (12·1–9·1)	1·3
Nitrazepam	30·2 (36·2–25·1)	1·2	26·0 (32·5–20·8)	1·3	23·0*	1·3	7·8 (9·1–6·6)	1·8

ED50 values with 95% confidence limits for loss of righting reflex in mice produced by the benzodiazepines in various vehicles. The formulations were injected intraperitoneally, each benzodiazepine being used in a concentration of 5 mg/ml of vehicle. Relative potencies (RP) (chlordiazepoxide = 1) are shown. The ED50 value for nitrazepam (*) is approximate as the probit line was non-linear (see text).

With each of the benzodiazepines, the rank order of the ED50 values varies with the vehicle used. The mean values decrease in the order, methylcellulose vehicle, DMSO, propylene glycol solvent and glycofurol solvent. The exception is diazepam, where similar ED50 values were obtained with propylene glycol solvent and glyco-

furol solvent, though both were markedly different from the values for diazepam in the other solvents.

The variation in the results obtained suggests that the potency of the benzodiazepines is influenced by the vehicle used. In experiments where the effects of the vehicles alone were tested, with the exception of the methylcellulose vehicle, each solvent produced a loss of righting reflex in mice. Table 2 shows ED50 values and slope functions for the solvents, expressed as ml/kg. Table 2 also shows the ED50 values for the drug-vehicle combinations, expressed as ml/kg, when the benzodiazepines were used at 5 mg/ml in the various vehicles. In this comparison it can be

Table 2. *ED50 values (ml/kg) for the vehicles and for the benzodiazepine (5 mg/ml) formulations.*

	Methylcellulose vehicle		DMSO		Propylene glycol solvent		Glycofurol solvent	
	ED50	Slope function	ED50	Slope function	ED50	Slope function	ED50	Slope function
Solvent alone	Greater than 30 ml/kg		14·8 (18·5–11·9)	1·7 (2·1–1·7)	9·6 (11·0–8·4)	1·6 (2·0–1·3)	3·3 (3·6–3·0)	1·3 (1·6–1·1)
Chlordiazepoxide HCl (5 mg/ml)	7·1 (7·4–6·7)	1·2 (1·3–1·1)	7·0 (8·0–6·1)	1·7 (2·0–1·4)	6·1 (8·4–5·2)	1·4 (1·7–1·2)	2·8 (3·5–2·3)	1·3 (1·4–1·2)
Diazepam (5 mg/ml)	5·1 (6·6–3·9)	2·1 (2·6–1·7)	3·7 (4·7–2·9)	1·9 (2·5–1·5)	0·57 (0·66–0·40)	1·6 (2·0–1·2)	0·59 (0·70–0·48)	1·5 (1·8–1·3)
Medazepam (5 mg/ml)	8·4 (10·5–6·6)	2·2 (2·2–1·8)	6·3 (6·9–5·7)	1·3 (1·6–1·1)	3·3 (3·6–3·0)	1·2 (1·3–1·2)	2·1 (2·4–1·8)	1·4 (1·5–1·1)
Nitrazepam (5 mg/ml)	6·6 (7·2–5·0)	1·9 (2·3–1·7)	5·2 (6·5–4·2)	1·8 (2·6–1·3)	4·6*	—	1·6 (1·8–1·3)	1·6 (2·0–1·2)

As in Table 1: ED50 values, slope functions and 95% confidence limits for the vehicles alone and for the benzodiazepine formulations expressed as ml/kg. The ED50 for nitrazepam in propylene glycol solvent (*) is approximate owing to non-linearity of the probit plot.

seen that the ED50 value for DMSO (14·8 ml/kg) is much higher than that obtained with each benzodiazepine-DMSO combination. With the propylene glycol solvent the ED50 value is 9·6 ml/kg, and this again is in excess of the values obtained with each of the drug-solvent combinations. The ED50 value for the glycofurol solvent (3·3 ml/kg) is similar to the values obtained when chlordiazepoxide HCl and medazepam were dissolved in this vehicle (2·8 and 2·1 ml/kg respectively). This suggests the possibility of an interaction between these drugs and the glycofurol solvent.

To evaluate the way in which these vehicles might influence the potency of the drugs, both the pharmacological and the physicochemical properties of the vehicle and the drug-vehicle combination must be considered. Gaddum (1953) described a method for evaluating the interaction between two drugs that have a similar pharmacological action. Using cartesian coordinates the ED50 value for one drug is plotted on one axis and the ED50 value for the second drug is plotted on the other axis (Points A & B in Fig. 1a). ED50 values for combinations of the two drugs are plotted in the field. Points above the horizontal line AC represent antagonism of drug A by drug B; points to the right of the vertical line BC represent antagonism of drug B by drug A, and points within the rectangle OACB represent synergism between the two drugs. If a point falls on either line AC or BC, no interaction is present at that ratio.

A plot evaluating the interaction between the water-soluble drug chlordiazepoxide HCl and the glycofurol solvent is shown in Fig. 1b. The broken line in this Figure and also in Fig. 1c and 1d represents a constant ratio of 5 mg of the benzodiazepine to 1 ml of the solvent, as used initially (Table 1). The ED50 for chlordiazepoxide HCl in water is 35·4 mg/kg, while that for glycofurol solvent alone is 3·3 ml/kg. The

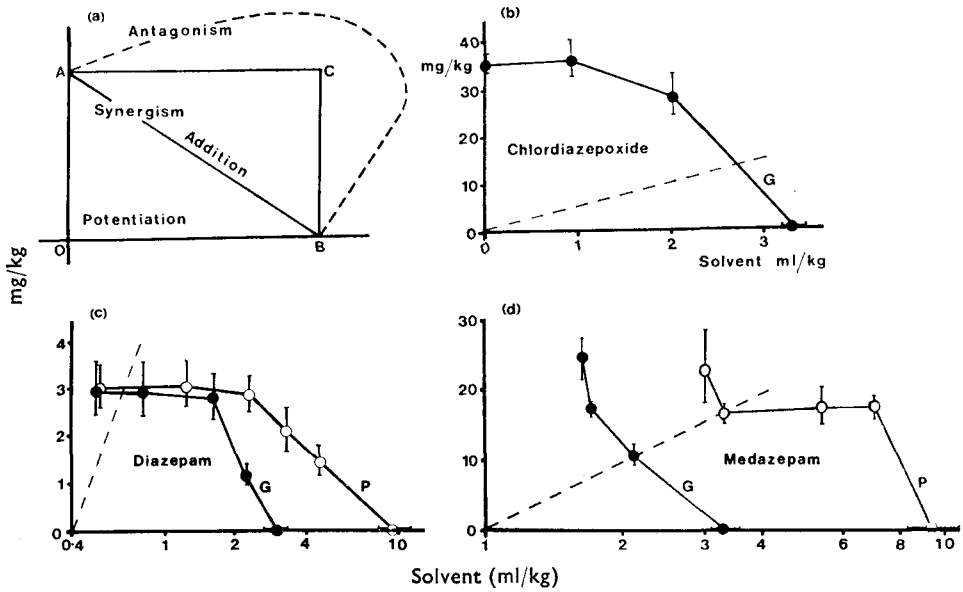

FIG. 1. (a) Diagrammatic representation (redrawn from Gaddum, 1953) of a method for evaluating interactions between two drugs (see text). ED50 values with 95% confidence limits for loss of righting reflex in mice produced by combinations of (b) chlordiazepoxide HCl, (c) diazepam and (d) medazepam with glycofurol solvent, G (●) and propylene glycol solvent, P(○). The broken lines in (b) (c) and (d) represent a constant ratio of drug to solvent of 5 mg/ml.

ED50 value for chlordiazepoxide HCl when administered with a constant dose of either 1 or 2 ml/kg of glycofurol solvent is shown. At 1 ml/kg of the glycofurol solvent, no interaction is apparent, while at 2 ml/kg synergism occurs, as indicated by a departure from horizontal. At a ratio of 5 mg/ml (broken line) marked synergism is apparent and the ED50 value quoted in Table 1 for the chlordiazepoxide HCl-glycofurol solvent combination is the result of synergistic action.

When similar plots are produced with diazepam (Fig. 1c), which is insoluble in water, it is not possible to obtain a point on the vertical axis. However ED50 values for diazepam with constant doses of 0·5, 1·0 and 2·0 ml/kg of both glycofurol solvent and propylene glycol solvent, lie on a horizontal line. This indicates that there is a range of drug-solvent ratios where interaction is absent. Extrapolation of the horizontal line may therefore give an estimated ED50 value of 2·9 mg/kg for diazepam. The broken line (ratio 5 mg/ml) passes through the horizontal component of both lines, indicating an absence of interaction with either propylene glycol solvent or glycofurol solvent at this drug-solvent ratio.

With medazepam, through the full range of solubility in the glycofurol solvent, no horizontal component is apparent (Fig. 1d) but with the propylene glycol solvent there is a horizontal component and extrapolation from this line gives an estimated ED50 of 16·5 mg/kg. It should be noted that the 5 mg/ml line passes through the horizontal component of this line indicating an absence of interaction with the propylene glycol solvent at this drug-solvent ratio. The upper point on each line in Fig. 1d represents a point where, using a fixed dose of solvent, difficulty was experienced in maintaining solubilization of the drugs. The result is an upward distortion of the line and a widening of the 95% confidence limits. This effect is possibly due

to precipitation of the drug at the site of administration and a resulting decrease in potency.

Difficulties were experienced when attempting to assess the ED50 values of nitrazepam in the presence of the propylene glycol solvent. Fig. 2 shows the probit lines obtained for nitrazepam with different doses of the propylene glycol solvent. As each line is non-linear, an ED50 value could not be calculated. However, the probit line obtained for nitrazepam in the methylcellulose vehicle (broken line, Fig. 2) lies close to the second upward deflection of each of the probit lines obtained in the presence of the propylene glycol solvent.

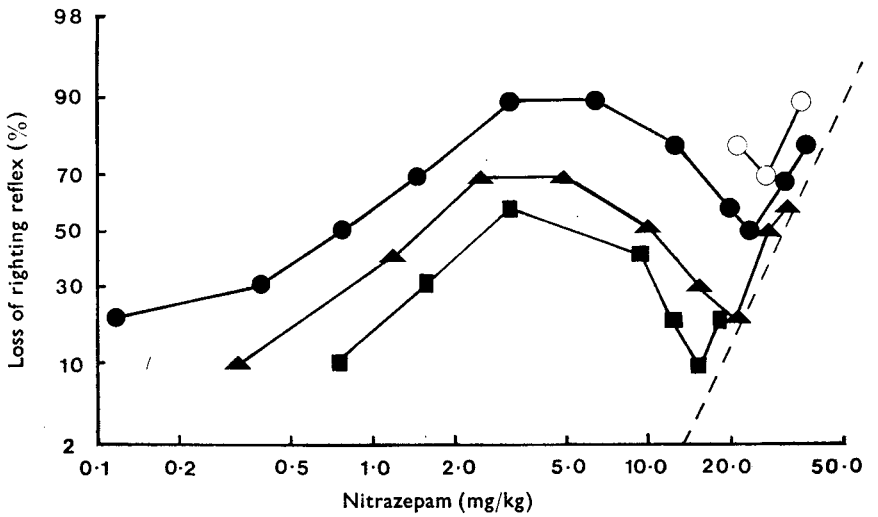

FIG. 2. Probit analysis showing the loss of righting reflex in groups of 10 mice produced by varying doses of nitrazepam in the presence of constant doses of the propylene glycol solvent (7 ml/kg, ○; 6 ml/kg, ●; 5 ml/kg, ▲; and 3 mg/kg, ■). The broken line to the right shows the position of the line of best fit of the probit plot for nitrazepam suspended in the methylcellulose vehicle.

As it was not possible to calculate the ED50 values for nitrazepam in the propylene glycol solvent, further experiments were made. It was found that if the dose of nitrazepam was kept constant for a given test and the amount of propylene glycol solvent was varied, ED50 values could be obtained for the solvent in the presence of the drug. The use of a constant dose of nitrazepam with a variable dose of the propylene glycol solvent does not alter the basic method of studying an interaction, but avoids extending the doses of nitrazepam into a range where multiple actions are observed. In Fig. 3 the interaction between nitrazepam and the propylene glycol solvent is shown. The vertical axis represents the various fixed doses of nitrazepam administered when various ED50 values for the propylene glycol solvent were obtained. The horizontal axis represents the actual concentration of nitrazepam in the propylene glycol solvent at each point. This method of plotting demonstrates the variation in potency of nitrazepam with varying strengths of solution, no horizontal component being present in the plot.

When ED50 values were obtained with constant doses of DMSO in combination with varying doses of nitrazepam, a plot of ED50 values against strength of solution was obtained (Fig. 3). It is apparent that DMSO, like propylene glycol solvent, is unsatisfactory as a solvent as there is no horizontal component to the line.

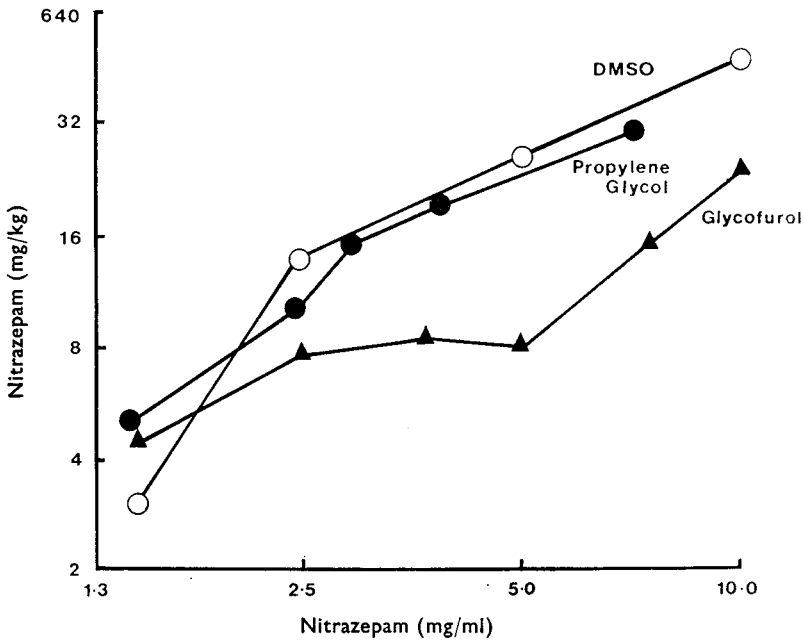

FIG. 3. Dose of nitrazepam required to produce loss of righting reflex in 50% of mice (ordinate) plotted against the concentration of this drug in various solvents (abscissa). In the case of DMSO (○) and the glycofurol solvent (▲), the values plotted on the ordinate are ED50 values for nitrazepam. In the case of the propylene glycol solvent (●) the points on the ordinate represent constant doses of nitrazepam administered in combination with varying doses of propylene glycol solvent.

When ED50 values were obtained for nitrazepam in the presence of the glycofurol solvent and plotted in the same way as for the other two solvents, a horizontal component was present (Fig. 3). Extrapolation of the horizontal component of this line gives an ED50 value for nitrazepam of 7·7 mg/kg.

DISCUSSION

Of the benzodiazepines considered, chlordiazepoxide HCl alone is water-soluble. In most animal tests, these drugs have been given by mouth. The insoluble drugs have usually been administered in some form of suspension (see Zbinden & Randall, 1967, for review). To produce the pharmacological effects under test, a drug must achieve a critical concentration in the biophase which surrounds the active sites involved. For the drug to pass from the site of administration to the site of action a number of criteria must be satisfied. The solvent must maintain solution of the drug *in vitro*; it must ensure continued solution of the drug at the site of administration; it must not impede the uptake of the drug into the circulation, and finally, it must not interfere with the drug at its site of action.

In the situation studied, with chlordiazepoxide HCl, all criteria are satisfied when the drug is given in water. If chlordiazepoxide is given with the solvent DMSO or other pharmacologically active vehicles, its action may be potentiated.

With diazepam, medazepam and nitrazepam, which are all insoluble in water, attention must be paid to the choice of vehicle. The use of an aqueous vehicle, containing suspending agents, results in consistently high ED50 values when the

responses are compared with those obtained with other solvents. This suggests that uptake into the circulation is slow. A probable explanation is slow dispersion of the drug from undissolved particles at the site of injection. The relative potencies obtained when using the methyl cellulose vehicle (Table 1) are similar to those given by Zbinden & Randall (1967) for oral administration of the drugs, where a similar pharmacological test was used (chlordiazepoxide HCl = 1·0; diazepam = 1·6; medazepam = 0·44 and nitrazepam = 0·84). The doses required were approximately ten times greater by mouth than parenterally. This similarity in relative potency probably indicates that absorbtion of the drugs from the lumen of the gastrointestinal tract and from the peritoneal surfaces involves a similar mechanism. The difference in the absolute potency is possibly the result of different overall rates of absorption.

When the solvent DMSO is used, similar ED50 values are obtained to those found with the methylcellulose vehicle. Precipitation of the water-insoluble drugs at the injection site is a probable explanation of these results, although inspection of the peritoneal cavity of the animals did not reveal any gross precipitation. DMSO, through hydrogen bonding, has an extremely high affinity for water (MacGregor, 1967). Contact with the body fluids leads to disruption of the anhydrous polymerization of DMSO and a rapid dispersion of this substance in the body (Rammler & Zaffaroni, 1967). This disruption of the anhydrous structure destroys the ability of DMSO to maintain solution of a non-ionized solute (Parker, 1965). Such an effect would result in precipitation of the benzodiazepines at the injection site. Thus DMSO is a totally inadequate solvent for the maintenance of solution of these drugs in contact with body fluids. Furthermore, DMSO has a variety of pharmacological actions that could interfere with the actions of the drugs (Leake, Rosenbaum & Jacob, 1967).

The propylene glycol solvent has relatively low biological activity and is therefore potentially suitable as a solvent for benzodiazepines that are insoluble in water. It is suitable for diazepam, and also for medazepam which has relatively low biological activity. The absence of constant ED50 values with different strengths of nitrazepam in the propylene glycol solvent, makes this particular drug-solvent combination of limited use.

The reversal of the depressant action of nitrazepam in the presence of the propylene glycol solvent is of considerable interest. Sternbach, Randall & Gustafson (1964) have described an excitatory action of nitrazepam when large doses are given by mouth. Finney (1947) has described a dose response curve similar to that obtained in the present experiments when the antifungal action of tetramethylthiuram was tested. Finney suggested that the non-linear response could be due to competition between a dissociated and an un-dissociated form of this compound, the result being a fall in potency in certain concentration ranges. Such an explanation could fit the effects observed with nitrazepam. However, the phenomenon might also be explained in terms of a differential depression of central excitatory and inhibitory neurons in the central nervous system. In the context of this paper, it appears that the effects observed are properties of the drug nitrazepam, and the solvent is merely modifying these.

The glycofurol solvent dissolves each of the benzodiazepines, but has the highest biological activity of the vehicles tested. It potentiates the action of medazepam

when the drug is given at the limit of solubility. This interaction makes the combination unsuitable if solvent effects are to be excluded.

Both diazepam and nitrazepam are more potent than medazepam and solutions may be prepared in which the potency of the drugs is not influenced by the presence of the solvent. Due to interactions, other solvents are unsatisfactory and the glycofurol solvent appears to be the vehicle of choice for nitrazepam in the situation studied.

Table 3. *ED50 values for benzodiazepines.*

	ED50	Slope function	Relative potency	Solvent*
Chlordiazepoxide HCl ..	35·4 (37·2–33·5)	2·1 (2·9–1·5)	1·0	Water
Diazepam	2·9 (3·5–2·4)	1·6 (1·8–1·3)	12·2	Glycofurol solvent or propylene glycol solvent
Medazepam	16·5 (17·8–15·3)	1·2 (1·3–1·1)	2·1	Propylene glycol solvent
Nitrazepam	7·7 (9·1–6·6)	1·6 (2·0–1·3)	4·6	Glycofurol solvent

ED50 values (mg/kg), slope functions, 95% confidence limits and relative potencies (chlordiazepoxide HCl = 1) are shown for chlordiazepoxide HCl, diazepam, medazepam and nitrazepam. To avoid interactions at solution strengths of 5 mg/ml.

Table 3 shows the ED50 values, with slope functions and 95% confidence limits, for each drug after solvent effects have been excluded. The vehicles needed to avoid drug-solvent interactions are shown in each case. Relative potencies are also shown (chlordiazepoxide HCl = 1).

These experiments show that the absolute and relative potencies of the benzodiazepines chlordiazepoxide HCl, diazepam, medazepam and nitrazepam, when administered parenterally, may be modified by the solvents used. These interactions can cause either a reduction or an enhancement of the actual potencies of the compounds. Glycofurol solvent, propylene glycol solvent, methylcellulose vehicle and DMSO have been used as vehicles for these drugs. The present experiments indicate that their biological activity and their limitations as solvents must be considered whenever they are used. A simple means of identifying solvent effects is to vary the amount of solvent used and to test for a change in the potency of the drug. If a change in potency is apparent, further testing is necessary to identify the nature of the effect.

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