of a particular component ratio. This discrepancy might be attributed to the fact that Sekine et al (1984) did not evaluate the action of the individual components, as discussed above. Besides, differences in technique of the animal experiments and the use of different antibiotics might contribute to the discrepancy.

The absorption promoting effect of GMO may be mediated by interference with the orientation of membrane phospholipids, as has been described for glycerylmonooleate (Muranushi et al 1981). Since GMO has been reported to solubilize cholesterol (Lillemoe et al 1982), an additional transcellular absorption-enhancing effect may be caused by extraction of the membrane stabilizing agent cholesterol from the epithelial membranes. The effectiveness of GMO suggests that further exploration of the absorption promoting properties of monoglycerides, should be interesting.

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4-Methylpyrazole alters phenobarbitone hypnotic concentrations in rats

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Abstract—The liver alcohol dehydrogenase inhibitor, 4-methylpyrazole, has been tested for its ability to change the hypnotic concentrations of phenobarbitone (phenobarbital) in rats. Following a single dose of 1 mmol kg^{-1} i.v., administered 60 min before phenobarbitone, 4-methylpyrazole shortened the onset time and reduced the dose of phenobarbitone required to produce loss of righting reflex. Consistent with this, phenobarbitone concentrations in serum (both total and free), brain and in cerebrospinal fluid at onset of hypnosis were about half in 4-methylpyrazole compared with saline-treated rats. These results suggest that acute 4-methylpyrazole pretreatment increases the central nervous system sensitivity to phenobarbitone and presumably other barbiturates; an effect apparently distinct from its inhibition of liver alcohol dehydrogenase.

Pyrazole and 4-methylpyrazole (4-MP) are potent competitive inhibitors of mammalian liver alcohol dehydrogenase (Li & Theorell 1969), with 4-MP being more useful clinically, presumably due to its low toxicity and high specificity against the enzyme (Inoue et al 1985).

Potentiation of the action of central nervous system (CNS) depressants by the pyrazoles has been taken as evidence for the involvement of liver alcohol dehydrogenase in their metabolism (Schultz & Weiner 1979). However, pyrazole potentiates the effect of trichloroethanol which is not a substrate for this enzyme (Owen & Taberner 1980). In addition both pyrazole and 4-MP increase sleep time in mice following chloral hydrate, pentobarbitone, barbitone, temazepam and halothane, but not diethyl ether (Taberner & Unwin 1987). In the same study, 4-MP produced a shortening of the latency to loss of righting reflex (LRR) following barbitone which is not metabolized. This suggests that the effect of these pyrazoles and in particular 4-MP is not specific to the inhibition of liver alcohol dehydrogenase and that it may potentiate the CNS activity of barbiturates via a pharmacodynamic mechanism.

The aim of the present study was therefore to examine in detail the reported interaction between 4-MP and barbiturate sensitivity. Specifically, the effect of acute 4-MP pretreatment on phenobarbitone concentrations at the onset of hypnosis or LRR, was examined. Phenobarbitone (instead of barbitone or pentobarbitone) was used since phenobarbitone is optically pure, has a long half life, thus minimizing possible 4-MP effect on its pharmacokinetics and since its cerebrospinal fluid (CSF) concentrations at onset of LRR reflect effective concentrations at its biophase or effector site (Danhof & Levy 1984). Rats (rather than mice) were used to allow a comprehensive investigation with measurement of both free (unbound) and total phenobarbitone concentrations in arterial serum, CSF and brain tissue at LRR.

Methods

Adult male Sprague-Dawley rats, purchased from a commercial supplier, were housed individually at 22–25°C on a normal 12 h light/dark cycle for a week before the study and had free access to rat chow and water, except during testing. One day before the study, all animals had a silastic cannula inserted into their right external jugular vein (Weeks & Davis 1964) during light ether anaesthesia, a procedure which takes 10–15 min to complete. All drugs (or saline) were administered via this cannula.

Two groups of rats were injected i.v. with 4-MP (1 mmol kg^{-1} as a 1 mmol mL^{-1} solution in saline) or saline (1 mL kg^{-1}) 60 min before the start of the barbiturate infusion. This dose of 4-MP has been used previously (Taberner & Unwin 1987) in mice and is comparable to the reported (Goldberg & Rydberg 1969) ED50 of 0·3 mmol kg^{-1}. Each animal was then infused with phenobarbitone (as sodium salt in saline) at 2·72 mg min^{-1} per rat until onset of LRR (animal unable to right itself when placed on its back); the infusion rate being chosen based on a previous comprehensive study of phenobarbitone pharmacodynamics in rats (Danhof & Levy 1984). To prevent any bias in the observation of the endpoint, the author assessed the onset of LRR while the time to LRR was kept independently by a student assistant. During the infusion the rats were placed on isothermal pads to prevent hypothermia which accompanies phenobarbitone infusion (Danhof & Levy 1984).

At onset of LRR, the infusion was stopped and samples of CSF (~50 μL by cisternal puncture), arterial blood (5–10 mL from abdominal aorta) and the whole brain (after decapitation) were obtained in that order. Phenobarbitone concentrations were determined using 5-ethyl-5-*p*-tolybarbituric acid (2 or 0·5 mg mL^{-1} in acetonitrile) as internal standard. To serum (100 μL) or CSF (25 μL) was added 10 μL of internal standard solution and 100 and 50 μL, respectively, of acetonitrile followed by HPL chromatography of the supernatant (50 μL). A mixture of brain tissue (1 g from one hemisphere), internal standard solution (100 μL) and acetonitrile (1 mL) was homogenized, centrifuged and chromatographed as for serum and CSF. Chromatographic conditions included use of a reversed phase C_{18} column with 50% methanol–water and 0·005 m KH_2PO_4 as mobile phase, 1 mL min^{-1} flow rate and UV detection at 254 nm; assay coefficient of variation was 2·5% between 50 and 400 mg L^{-1} or mg kg^{-1} and detection limit was 1 mg L^{-1}. Phenobarbitone serum protein binding determinations were done by equilibrium dialysis (pH 7·4 buffer, 6 h equilibration time) followed by chromatography.

Phenobarbitone doses (onset times × infusion rate) and concentrations between the two groups were tested for statistical significance using the nonparametric Mann Whitney U test.

Results

Bolus i.v. injections of 4-MP produced a transient (15–30 s duration) ataxia in all treated animals but no other overt signs of CNS depression (or stimulation) were observed during the 60 min pretreatment period.

4-MP pretreatment halved the time to onset of LRR and significantly reduced the absolute (mg) and relative (mg kg^{-1}) phenobarbitone cumulative dose required to produce LRR (Table 1). Consistent with this, phenobarbitone concentrations in serum, brain and most importantly in CSF at LRR were about half that noted in saline treated controls. 4-MP did not affect the serum protein binding of phenobarbitone.

Table 1. Effect of 4-methylpyrazole (4-MP) on onset times, doses and concentrations of phenobarbitone at onset of loss of righting reflex (LRR) in rats.

Item	Control	4-MP-treated
Body weight (g)	319±11	316±13
Infusion time (min)	18±5	10±1*
Cumulative dose (mg)	48±12	27±3*
Cumulative dose (mg kg^{-1})	150±41	84±8*
Serum total concn. (mg L^{-1})	177±37	138±16**
Serum free (unbound) concn. (mg L^{-1})	103±19	78±11**
CSF concn (mg L^{-1})	50±19	29±4***
Brain concn (mg kg^{-1})	104±18	76±15**
Serum free (unbound) fraction	0·588±0·068	0·565±0·042

Rats were pretreated with 4-MP (1 mmol kg^{-1} i.v.) or saline 60 min before phenobarbitone infusion at 2·72 mg min^{-1} to LRR. Values represent mean ± s.d., n = 9, 10 in control and treated groups, respectively, except for CSF concns where n = 6 for each group. 4-MP pretreatment < saline control, * $P < 0·0002$; ** $P < 0·005$; *** $P < 0·01$.

Discussion

The results of the present study indicate that acute pretreatment with 4-MP increases the CNS sensitivity to phenobarbitone in rats as evidenced by lower phenobarbitone concentrations at the onset of LRR. This effect appears to be pharmacodynamic rather than pharmacokinetic in origin since 4-MP not only changes the serum but also the CNS (both brain and CSF, respectively) concentrations and does not affect its free (or unbound) concentration in serum. These results are in agreement with, and suggest a pharmacodynamic basis for, the prolongation of the CNS effect of a number of depressants by 4-MP as noted previously in mice (Taberner & Unwin 1987).

Phenobarbitone was chosen as the agent to investigate the reported interaction between barbiturates and 4-MP since it has a long half-life in rats; its major 4-hydroxy metabolite is pharmacologically inactive and since CSF phenobarbitone most closely reflects it hypnotic concentrations at the site(s) of CNS depression (Danhof & Levy 1984). Thus examination of phenobarbitone CSF concentrations at LRR and any effect of 4-MP thereon apparently reflects a true pharmacodynamic (concentration-hypnotic activity relationship) effect. On this basis, 4-MP increases the sensitivity to phenobarbitone in rats. It is not known how 4-MP produces this effect or whether it acts via the barbiturate site of the benzodiazepine-G ABA receptor complex.

4-MP is widely used in alcohol research and as therapy in methanol and ethylene glycol toxicity. The present results suggest that a single dose of 4-MP is likely to potentiate the effects of concomitantly administered barbiturates such as phenobarbitone.

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