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Non-specific prolongation of the effects of general depressants by pyrazole and 4-methylpyrazole

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The liver alcohol dehydrogenase inhibitors, pyrazole and 4-methylpyrazole, have been tested for their ability to prolong drug-induced sleep times in mice. Both drugs (at 1 mmol kg^{-1} i.p.) prolonged the duration of loss of righting reflex following chloral hydrate, pentobarbitone, barbitone, temazepam and halothane, but not diethyl ether. This suggests that the effects of these pyrazoles are not specific to the inhibition of liver alcohol dehydrogenase.

Pyrazole and 4-methylpyrazole (4-MP) have been recognized for many years as potent competitive inhibitors of mammalian liver alcohol dehydrogenase (LAdH) (Li & Theorell 1969). In fact, the potentiation of general depressants by pyrazole has been taken as evidence for the involvement of LAdH in their metabolism (see Taberner et al 1972; Schultz & Weiner 1979). Both pyrazole and 4-MP have been shown to prevent ethylene glycol (Chou & Richardson 1978) and methanol (Blomstrand et al 1979) toxicosis, and because of the apparent low toxicity and high specificity of 4-MP as an LAdH inhibitor it has been increasingly used in man (Salaspuro et al 1977; Lindros et al 1981; Inoue et al 1985).

However, pyrazole can potentiate trichloroethanol, which is not a substrate for LAdH (Owen & Taberner 1980). We have therefore investigated both pyrazole and 4-MP for their ability to potentiate the depressant effects of a range of CNS acting drugs.

Methods

Adult LACG mice from a colony inbred in the Medical School were housed at 20–22 °C on a normal light/dark cycle and had free access to food (Oxoid breeding diet) and water. Drugs were made up in 0·9% (w/v) NaCl (saline) except for halothane, ether and temazepam which were suspended in arachis oil (BP). Drugs were administered by intraperitoneal injection at 0·05 mL/10 g body weight, except for ether and halothane which were given at 0·1 mL/10 g body weight. Temazepam free base was donated by Farmitalia Carlo Erba Ltd.; all the other drugs used were from commercial sources. The barbiturates were given as the sodium salts.

All experiments were performed between 1000 and 1400h. Groups of 8 mice were injected with pyrazole (1 mmol kg^{-1}), 4-methylpyrazole (4-MP, 1 mmol kg^{-1}), or saline 60 min before the dose of anaesthetic.

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The loss of righting reflex was assessed by an independent observer. The duration of the loss of righting reflex was defined as the time from when the animal became unable to right itself for 15 s after being placed on its back to the time at which it could right itself 3 times within 15 s. Differences in latency of duration of loss of righting reflex between groups were tested for statistical significance using Student's *t*-test.

Results

Preliminary experiments were performed to find doses of the anaesthetics that would produce loss of righting reflex in all the animals tested. They were (in mg kg^{-1}): ethanol 4000, chloral hydrate 300, pentobarbitone 50, barbitone 200, temazepam 50, halothane 1000, and ether 1000. The latencies to loss of righting reflex varied from 2·4 ± 0·4 min (mean + s.e.m.) for ethanol, to 34·4 ± 4·8 min for barbitone. Pyrazole and 4-MP at equimolar doses produced no detectable signs of sedation when administered alone. Pretreatment with pyrazole or 4-MP did not significantly alter the latency to loss of righting reflex except in the case of barbitone where 4-MP produced a significant shortening of the latency to 23·2 ± 2·2 min ($P < 0·02$).

The duration of loss of righting reflex after the various drug treatments is shown in Fig. 1. Pyrazole produced a significant increase in the duration of loss of righting reflex after all the drugs except ether. 4-MP significantly increased the duration after all the drugs except ether and halothane. (The latter was increased but not significantly.)

Discussion

Ethanol and chloral hydrate are almost completely metabolized by LAdH, which is inhibited by pyrazole and 4-MP. Pentobarbitone and temazepam are metabolized by the cytochrome P450 system in the liver (Breimer 1977). Halothane is largely excreted unchanged, although a proportion is metabolized (Cohen 1971). Barbitone and ether are not metabolized to any significant extent. It would appear, therefore, that both pyrazole and 4-MP can potentiate drugs which are not inactivated by LaDH or even, in the case of barbitone, metabolized at all.

The dose of pyrazole was chosen since it has been reported to inhibit ethanol disappearance in mice by 75% following 1 g kg^{-1} ethanol (Gessner & Dien 1970), the ED50 for inhibition of ethanol metabolism being

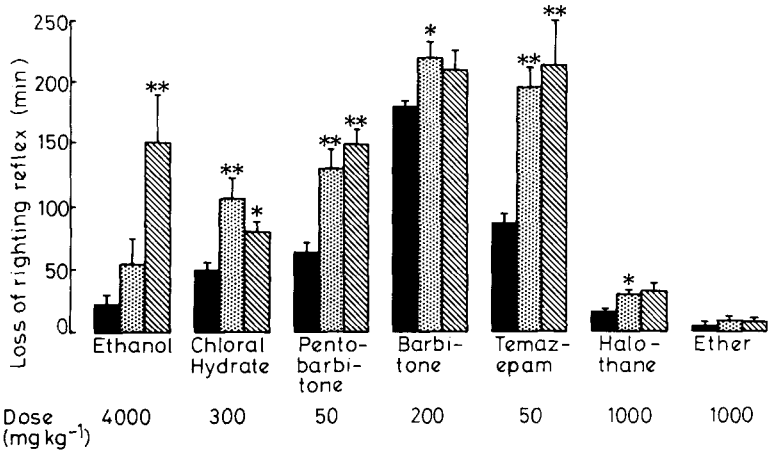

FIG. 1. Duration of loss of righting reflex (min) in mice produced by CNS depressants following pretreatment with saline (black), 1 mM pyrazole (spotted) or 1 mM 4-methylpyrazole (hatched). Results are means ± s.e.m., n = 8. Drug pretreatment > saline control, $*P < 0.05$; $**P < 0.01$.

somewhat lower in the mouse (0·15 mmol kg^{-1}) than in the rat (0·3 mmol kg^{-1}; Goldberg & Rydberg 1969). However, there are some puzzling discrepancies between the inhibitory potency of the pyrazoles in-vitro and in-vivo. The K_i for 4-MP against rat LAdH in-vitro is $1·3 \times 10^{-8}$ M (Dahlblom & Tolf 1974) and against mouse LAdH $2·2 \times 10^{-8}$ M (Taberner, unpublished observation). The IC50 can be calculated to be of the order of $3·4 \times 10^{-5}$ M at a blood alcohol concentration of 10^{-1} M (Cheng & Prusoff 1973). This is well below the blood concentration of $0·2 \times 10^{-3}$ M 4-MP recorded following a dose of 50 mg kg^{-1} in the rat (Blomstrand et al 1980). The circulating level of 4-MP following a dose sufficient to inhibit alcohol metabolism is therefore well in excess of that calculated to inhibit LAdH. 4-MP is a more potent inhibitor of LAdH than pyrazole and yet the LAdH activity measured in rat liver following chronic dosing with 0·425 mmol kg^{-1} day^{-1} was still 40% of control (Blomstrand et al 1980).

Both pyrazole and 4-MP are widely used in alcohol research, but the toxicity of pyrazole precludes its use in man (Lelbach 1969). However, 4-MP is finding increasing use in clinical research as a therapy against methanol poisoning (Blomstrand et al 1979), for preventing acute acetaldehyde toxicity in alcohol-sensitive subjects (Inoue et al 1985), and the disulfiram reaction in alcoholics (Lindros et al 1981). The present results suggest that 4-MP should not be regarded as a specific inhibitor of LAdH, and that the potentiation of the effects of concomitantly administered benzodiazepines or barbiturates is likely following doses of 4-MP.

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