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METABOLIC BASIS FOR THE SUPRA-ADDITIVE EFFECT OF THE ETHANOL-DIAZEPAM COMBINATION IN MICE

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1 The comination of ethanol and diazepam elicits additive or supra-additive pharmacological effects in animals and humans. Since the mouse appeared to be a reasonably good animal model for man, this species was examined to determine if a biochemical basis for these effects could be established.

2 The effect of ethanol (3 g/kg, orally), administered 0.5 h before [^{14}C]-diazepam (5 mg/kg, orally), on the pharmacokinetics of diazepam in male Swiss Webster mice was examined.

3 At 4, 6, 8 and 12 h after dosing, blood levels of ^{14}C were higher in ethanol pretreated mice than in vehicle pretreated controls. A tissue distribution study indicated that ethanol had similar effects on levels of ^{14}C in most tissues at 2, 6 and 12 hours.

4 Both pretreated and control mice eliminated approximately 25% of the dose of isotope in the urine and 50% in the faeces by 48 hours.

5 Biliary excretion was the major route of clearance in mice in which the bile ducts had been cannulated and ethanol reduced excretion at all time periods examined up to 12 hours.

6 Oxazepam (the glucuronide in bile) and desmethyl diazepam were the major metabolites identified in bile, plasma and brain. Ethanol reduced oxazepam levels but increased desmethyl diazepam levels, suggesting that 3-hydroxylation of desmethyl diazepam was inhibited by ethanol.

7 The accumulation of the pharmacologically active desmethyl diazepam in the brains of ethanol pretreated mice offers an explanation for the supra-additive effect of the ethanol-diazepam combination on motor coordination.

Introduction

Benzodiazepines have been used in the medical and dental field, because of their amnesic, sedative and muscle relaxant properties, for the symptomatic relief of tension and anxiety states. Recent reports have shown that their use has superseded that of barbiturates, making them the most commonly prescribed class of drugs (Shader, Greenblatt, Salzman, Kochansky & Harmatz, 1975; Kesson, Gray & Lawson, 1976). The metabolism of diazepam, one of the first marketed benzodiazepines, has been extensively studied in animals and man (Garattini, 1969; Schreiber, 1970; Zingales, 1973). The major diazepam degradation pathways are N_1-demethylation, C_3-hydroxylation and *para*-hydroxylation of the 5-phenyl ring (Figure 1); all except the latter leading to pharmacologically active derivatives (Garattini, 1969; Randall & Kappell, 1973).

Initial interaction studies carried out by Dundee & Isaac (1970) suggested that diazepam has no effect upon the blood concentration or dose of ethanol required to induce sleep, whereas chlorodiazepoxide was found to be an antagonist to ethanol. Other studies have not confirmed these findings but indicated that the combination of alcohol and diazepam elicits additive or supra-additive effects (Vapàatalo & Karppanen, 1969; Linnoila & Mattila, 1973a; Linnoila & Hakkinen, 1974; Mehar, Parker & Tubas, 1974; Morland, Setekleiv, Haffner, Stromsaether, Danielsen & Holst-Wethe, 1974). The basis for these additive or supra-additive effects of ethanol and diazepam in man has not been established. However, in a recent study from this laboratory it was found that ethanol elevated plasma and brain levels of diazepam by approximately 6-fold in the rat (Whitehouse, Paul, Coldwell & Thomas, 1975). When extrapolating these data to man, one is immediately confronted with the fact that the major route of metabolism in the rat is hydroxylation of the 5-phenyl ring, a pathway non-existent or of minor significance in man. Mouse and man share common major metabolic pathways for the biotransformation of diazepam (M_1-demethylation and C_3-hydroxylation; Schreiber, 1970). The effect of ethanol on the fate of diazepam was examined in the mouse to determine if a supra-additive or potentiating

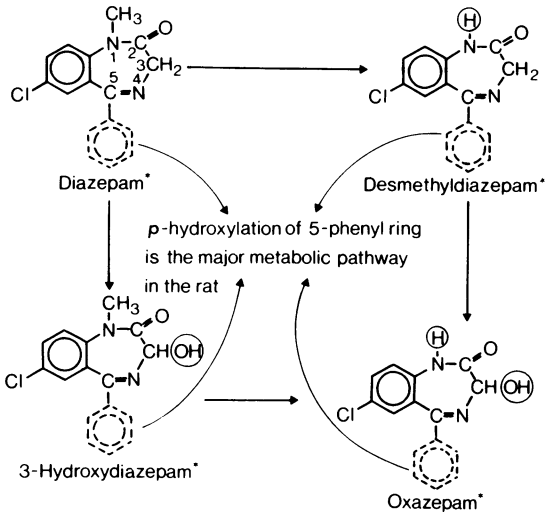

Figure 1 Major routes of diazepam biotransformation.

effect of diazepam in the presence of ethanol could be correlated with changes in the distribution and/or metabolism of diazepam.

Methods

Animal treatment

Male Swiss Webster mice (24–32 g) were acclimatized to the laboratory environment for at least one week before each experiment and food and water were supplied *ad libitum*. Before each experiment animals were fasted overnight (16 h) but allowed free access to water. Before (0.5 h) receiving diazepam (5 mg/kg, orally) mice were either given water 20 ml/kg orally (controls) or an aqueous (3.26 M) solution of ethanol equivalent to 3 g/kg. In the radioisotopic experiments [^{14}C]-diazepam labelled in the 5 position was given orally at a dose of 5 mg, 20 μCi/kg.

Common bile ducts were cannulated with heat-stretched polyethylene tubing (PE-10) while animals were under halothane anaesthesia. Mice were allowed 1 h following surgery to recover and were then dosed as described above, except that the dose of radiolabel was increased (5 mg, 40 μCi/kg, orally). Temperatures were monitored by rectal thermistor probes and body temperature maintained with heating lamps.

Sample collection and preparation

Blood (5–50 μl) collected between 15 min and 48 h, tissues (50–100 mg of liver, heart, kidney, muscle, omental fat and brain), fluids (blood 10 μl, plasma 25 μl, and bile 5 μl) and the gastrointestinal tract (g.i. tract) and contents (200 μl of an aqueous homogenate made to 15 ml with distilled water) collected at 2, 6, 12 and 24 h from 5 control and 5 pretreated mice following [^{14}C]-diazepam administration, were digested at room temperature overnight with Soluene-100 (Packard Instrument Canada Ltd., Montreal Canada). Bile samples (5 μl) collected between 30 min and 12 h from mice with cannulated bile ducts were similarly processed. With the exception of the g.i. tract and contents which required 2 ml of Soluene-100, digestion was carried out with 1 ml of solubilizer. Samples were bleached with hydrogen peroxide and slight heating. At the end of the decolourization treatment, excess hydrogen peroxide was removed by heating and the resulting solution neutralized (acetic acid, 0.4 ml) and treated with water (1.5 ml) and Aquasol (15 ml, New England Nuclear, Boston Mass. 02118). No loss of [^{14}C]-diazepam radioactivity was encountered during the bleaching procedure.

Urine (200 μl), collected for 48 h from 3 control and 3 pretreated groups of 8 animals each, was assayed for radioactivity in Aquasol (15 ml). Faeces from the same mice were collected and suspended in 100 ml of distilled water. Aliquots (1 ml) were air dried, digested overnight and then treated with ethanol (0.25 ml) to prevent frothing during the bleaching process.

Radioactivity in plasma and brain samples was extracted with octanol and ethyl acetate, respectively and the percentage extraction of ^{14}C determined as described previously (Whitehouse *et al.*, 1975).

Unless otherwise indicated, all samples were assayed for radioactivity by liquid scintillation counting using 15 ml of a toluene-based scintillation fluid (Liquifluor, New England Nuclear, Boston, Mass.) and a Nuclear-Chicago Mark II or Isocap 300 liquid scintillation counter as described by Thomas, Coldwell, Solomonraj, Zeitz & Trenholm (1972).

Separation of diazepam and its metabolites

The nature of the radioactivity in plasma and brain extracts was examined by ascending thin-layer chromatography (TLC) on silica gel (Analab HF 250 micron plates) employing the chloroform: *n*-heptane:ethanol (10:10:1) system described by Schwartz & Carbone (1970). Developed TLC plates were scraped with an automatic TLC zonal scraper (Snyder-Kimble, Analabs Inc.) into serial 3 mm sections and transferred to vials containing 10 ml Aquasol. Native bile and bile treated for 16 h with Ketodase (β-glucuronidase, Warner-Chilcott) were similarly examined by TLC. In addition, samples from individual mice were pooled to give composite samples of bile, plasma and brain which were Ketodase-treated as before and separated by two-dimensional TLC (system A, chloroform:*n*-heptane:ethanol, 10:10:1; system B, isopropanol:ammonia, 20:1).

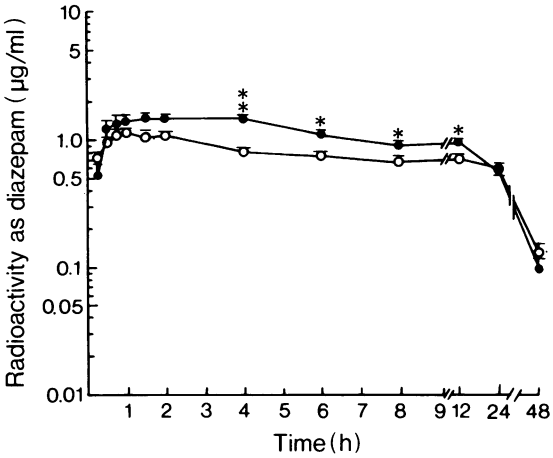

Figure 2 Time course of blood radioactivity in mice following vehicle (○) or ethanol (3 g/kg, orally; ●) pretreatment, 0.5 h before [^{14}C]-diazepam (5 mg/kg, orally) administration. Values are means from five animals and are expressed as μg of diazepam per ml of blood. Vertical lines show s.e. means. *$P<0.05$; **$P<0.01$.

Oxazepam isolated from pooled bile samples was unequivocally identified by mass spectrometry. Pooled bile samples were extracted with diethyl ether, the extract was concentrated, spotted on silica gel plates and developed in system A with authentic standards. Silica gel in the area corresponding to oxazepam was scraped from the plate, extracted with diethyl ether and the extract rechromatographed with authentic standards using system B. Silica gel in the area corresponding to oxazepam was again re-extracted with diethyl ether. A mass spectrum was obtained by the direct probe insertion method at an electron voltage of 80 eV and a source temperature of 110°C on a low resolution mass spectrometer (Hitachi-Perkin-Elmer model RMS-4). Results were confirmed with a high resolution mass spectrometer (Varian MAT 311A) set on a fixed mass and operated at a source temperature of 250°C and electron voltage of 70 eV.

Motor coordination

The rotacone, a modified rotarod, developed by Jacobsen (Danish Patent No. 117598, 1970) and described by Christensen (1973), was used to determine the effect of diazepam (5 mg/kg, orally), ethanol (3 g/kg, orally) and the diazepam-ethanol combination on animal motor coordination. Three groups of 8–9 mice were used in a cross-over design experiment and an interval of one week was allowed between each treatment. Motor coordination was examined at 0.5, 2, 4, 6 and 12 h after treatment with diazepam by the method of Christensen (1973). Results are expressed as activity ratios (AR):

$$AR = \frac{\text{Performance after drug administration}}{\text{Performance immediately before drug administration}}$$

Statistical analysis

Data were tested for statistical significance by Student's t test with $P<0.05$ being considered significant. In those cases in which percentages were compared, arc-sin transformed data were used (Snedecor & Cochran, 1967).

Results

Effect of ethanol pretreatment on the distribution and excretion of [^{14}C]-diazepam-derived radioactivity

Pretreatment with ethanol elevated blood concentrations of radioactivity at 4, 6, 8 and 12 h but did not statistically alter the area under the concentration–time curve (control, 25.20 ± 2.34 μg/ml × h; pretreated, 28.91 ± 1.69 μg/ml × h) or the $T_{\frac{1}{2}}$ (calculated from 12–48 h data) for elimination (control, 14.19 ± 1.48 h; pretreated, 10.96 ± 0.72 h; Figure 2).

Ethanol pretreatment increased the concentration of radioactivity from orally administered [^{14}C]-diazepam in several organs and tissues but reduced the radioactivity recovered in the bile (Table 1). Muscle, fat and heart tissues also contained more radioactivity in ethanol pretreated mice than in corresponding controls. Quantitation of the radioisotope in the g.i. tract (and contents) collected from vehicle pretreated mice indicated that 69.2 ± 4.1, 67.1 ± 2.6 and 47.7 ± 2.1% of the dose administered could be accounted for in the g.i. tract at 2, 6 and 12 h respectively. Corresponding samples collected from ethanol pretreated mice gave lower percentages (37.6 ± 2.5 ($P<0.001$), 52.4 ± 3.8 ($P<0.02$) and 59.9 ± 4.9). By 24 h all statistical differences between control and pretreated mice in the distribution of ^{14}C had disappeared.

Approximately 25% of the dose of radioactivity was eliminated from the body in the urine in 48 h and only at 12 h could a significant inhibitory effect of ethanol on the urinary excretion of ^{14}C be demonstrated (Figure 3). The major route of ^{14}C elimination was in the faeces (Figure 3), and this was attributed to biliary excretion. This was confirmed by examining the biliary excretion of radioactivity in mice with cannulated bile ducts (Figure 4). Approximately 65–75% of the dose administered to vehicle and ethanol pretreated mice was excreted in the bile in 12 h and at all time periods examined ethanol pretreatment statistically reduced biliary elimination of radioactivity (Figure 4).

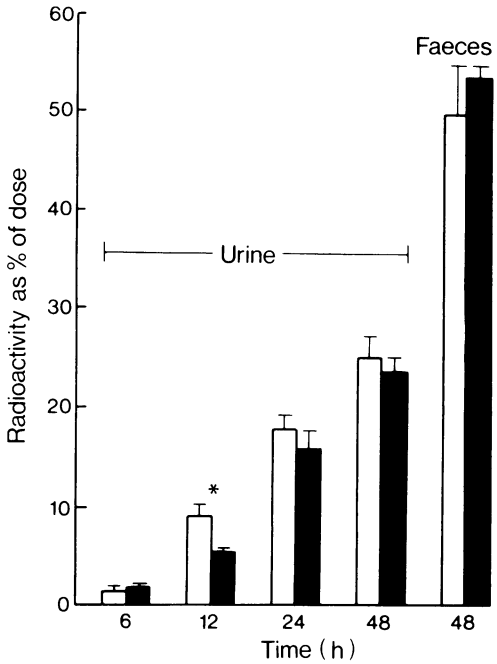

Figure 3 Cumulative urinary and faecal excretion of radioactivity by mice pretreated with vehicle (open columns) or ethanol (3 g/kg, orally, solid columns) 0.5 h before[^{14}C]-diazepam (5 mg/kg, orally). Values are means from three groups of 8 mice each. Vertical lines show s.e. means. * $P < 0.05$.

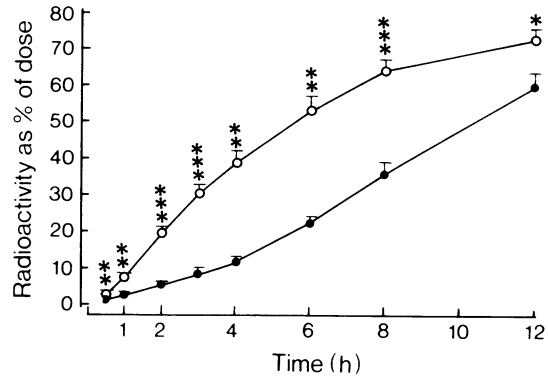

Figure 4 Cumulative biliary excretion of radioactivity by mice pretreated with vehicle (O) or ethanol (3 g/kg, orally, ●) 0.5 h before [^{14}C]-diazepam (5 mg/kg, orally) administration. Values are means from 4 animals. Vertical lines show s.e. means. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Metabolites of [^{14}C]-diazepam in bile, plasma and brain of vehicle and ethanol pretreated mice

Three major areas of radioactivity were separated by TLC in system A of bile samples collected during the first 2 h after giving [^{14}C]-diazepam (Table 2). Ethanol pretreatment reduced significantly the percentage radioactivity that remained at or near the origin (area I). Chromatography of Ketodase-treated biles indicated that the major metabolite(s) of area II was excreted as a glucuronide and two-dimensional TLC (Figure 5) suggested it was oxazepam. Ethanol pretreatment also reduced significantly the excretion of this metabolite. Mass spectrometry of an extract of the tentatively identified oxazepam spot gave a fragmentation pattern similar to that of authentic oxazepam (m/e peaks at 286, 268, 259, 257, 239, 233, 229, 214, 205, 177, 151, 104, 77, and 51) and high resolution mass spectrometry gave a mass (286.0505 ± 0.0006) comparable to the theoretical mass of oxazepam (286.0509). An examination of samples of bile

Table 1 Tissue distribution of radioactivity following [^{14}C]-diazepam (5 mg/kg, orally) administration to vehicle and ethanol (3 g/kg, orally) pretreated mice

			Time			
Tissue	*Pretreatment*	n	*2 h*	*6 h*	*12 h*	*Units*
Plasma	Vehicle	5	1.35 ± 0.15	0.72 ± 0.10	0.48 ± 0.05	μg/ml
	Ethanol	5	1.89 ± 0.11*	1.29 ± 0.17*	0.63 ± 0.04*	μg/ml
Brain	Vehicle	5	2.10 ± 0.17	1.07 ± 0.12	0.77 ± 0.08	μg/g
	Ethanol	5	4.38 ± 0.30***	2.88 ± 0.37**	1.26 ± 0.13**	μg/g
Kidney	Vehicle	5	3.53 ± 0.31	2.25 ± 0.27	1.67 ± 0.19	μg/g
	Ethanol	5	6.87 ± 0.55***	5.08 ± 0.51**	2.43 ± 0.30*	μg/g
Liver	Vehicle	5	9.62 ± 0.55	6.28 ± 0.67	3.85 ± 0.44	μg/g
	Ethanol	5	12.53 ± 0.71**	10.78 ± 1.08*	5.47 ± 0.54*	μg/g
Bile	Vehicle	3	0.15 ± 0.02	0.34 ± 0.04	0.31 ± 0.02	mg/ml
	Ethanol	4	0.04 ± 0.01**	0.17 ± 0.03**	0.23 ± 0.03	mg/ml

All values (means ± s.e. mean) are expressed as μg or mg of diazepam per g or ml.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; when compared with corresponding results of vehicle pretreated mice.

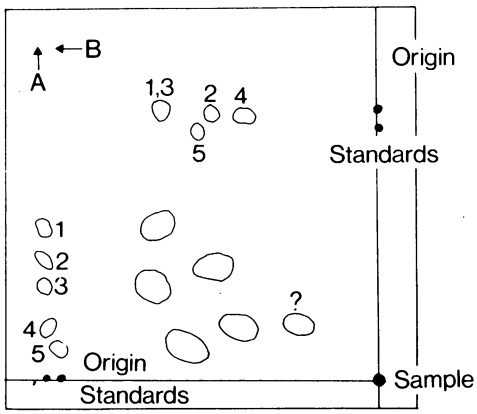

Figure 5 Typical two-dimensional thin-layer chromatographic separation of radioactivity of bile obtained after the administration of [^{14}C]-diazepam on silica gel HF 250 micro plates. Authentic standards with their respective R_Fs in system A (chloroform:*n*-heptane:ethanol, 10:10:1) and system B (isopropanol:ammonia, 20:1) were: 1 = diazepam, 0.35, 0.58; 2 = 3-hydroxy diazepam, 0.26, 0.40; 3 = desmethyldiazepam, 0.19, 0.58; 4 = oxaze pam, 0.10, 0.31; 5 = 5-phenyl-*p*-hydroxy desmethyl diazepam, 0.05, 0.43.

obtained up to 4, 8 and 12 h indicated that the distribution of radioactivity between the different areas resembled that seen at 2 hours.

Two-dimensional TLC of 12 h bile samples with authentic standards, as shown in Figure 5, indicated that radioactivity could be further resolved. On the basis of migration in two solvent systems with authentic standards, area I contained a minor metabolite, 5-phenyl-*para*-hydroxydesmethyl diazepam, which accounted for 6.6 ± 0.7 and 5.2 ± 0.5% of the dose administered to vehicle and ethanol pretreated mice respectively. Area II contained oxazepam (42.2 ± 1.3 and 36.4 ± 2.4% of the dose in control and ethanol pretreated mice, respectively) and also an unknown component which represented less than 2.5% of the dose in both groups. Area III contained two metabolites which migrated similarly to desmethyldiazepam and 3-hydroxydiazepam; control and ethanol pretreated mice respectively excreted 3.6 ± 0.6% and 1.6 ± 0.4% ($P < 0.05$) of the administered dose as desmethyldiazepam and 0.8 ± 0.2% and 0.8 ± 0.3% as 3-hydroxydiazepam.

Thin layer chromatography of plasma and brain extracts using system A indicated that oxazepam and desmethyl diazepam were the major sources of radioactivity in the plasma (>65%) and brain (>85%). Using pooled extracts of plasma and brain and two-dimensional TLC with authentic standards this observation was confirmed. Ethanol pretreatment significantly increased the octanol extractability of radioactivity in plasma at 2 h (75.1 ± 4.0 to 94.7 ± 0.5%) and TLC of these extracts indicated that plasma levels of oxazepam were reduced whereas desmethyldiazepam levels were significantly elevated (Table 3). Chromatographic examination of ethyl acetate extracts of brain (>98% recovery) showed that brain concentrations of oxazepam and desmethyl diazepam mirrored the results obtained from corresponding plasma samples, with the exception, that concentrations were approximately 2-fold greater (Table 3).

Effect of ethanol pretreatment on motor coordination in mice following diazepam administration

Oral administration of ethanol or diazepam alone did not significantly alter the performance of mice on the 'rotacone' from control values obtained immediately before dosing (Figure 6). However, the combination of ethanol and diazepam produced a supra-additive effect lasting for at least 6 hours. By 12 h the responses elicited from ethanol, diazepam and the

Table 2 Thin-layer chromatographic separation of radioactivity from bile obtained for 2 h following [^{14}C]-diazepam (5 mg/kg, orally) administration to vehicle and ethanol (3 g/kg, orally) pretreated mice

R_F range	Pretreatment	Native bile	Ketodase-treated bile
Area I 0.00–0.07	Vehicle	19.17 ± 1.63	9.24 ± 1.07
	Ethanol	5.35 ± 0.91***	1.31 ± 0.21***
Area II 0.07–0.11	Vehicle	0.29 ± 0.06	7.20 ± 0.78
	Ethanol	0.10 ± 0.01	1.77 ± 0.36**
Area III 0.12–0.22	Vehicle	0.14 ± 0.04	3.18 ± 0.45
	Ethanol	0.11 ± 0.05	2.53 ± 0.58

All samples were chromatographed on silica gel HF 250 micro plates using chloroform:*n*-heptane:ethanol (10:10:1). Values given are radioactivity recovered in the total bile over 2 h expressed as mean percentages of the dose ± s.e. mean from 4 mice.
** $P < 0.01$; *** $P < 0.001$ when compared with vehicle pretreated mice.

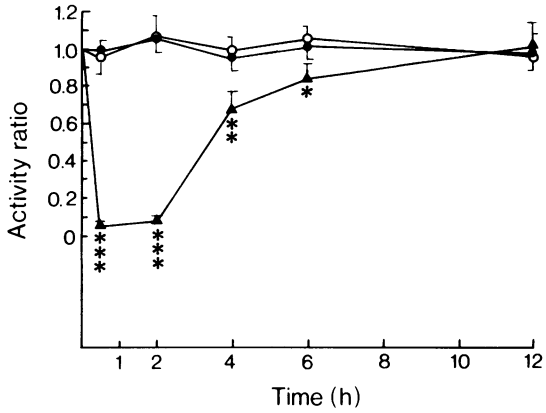

Figure 6 Effect of diazepam (5 mg/kg, orally, ○), ethanol (3 g/kg, orally, ●) and the combination (▲) on animal coordination expressed as activity ratios (response of animal on the rotacone after treatment divided by response before treatment). Values are means from 26 mice used in crossover designed experiments. Vertical lines show s.e. mean $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

combination of ethanol and diazepam were comparable.

Discussion

The supra-additive or potentiating effect of the ethanol-diazepam combination observed in mice (Figure 6) has been reported previously in man (Linnoila & Mattila, 1973a, b; Linnoila, Saario & Maki, 1974; Linnoila & Hakkinen, 1974; Morland *et al.*, 1974) and animals (Vapàatalo & Karppanen, 1969) but a pharmacological basis for these effects has not been established. Vuorinen, Heinonen & Rosenberg (1976) reported a study on the effect of ethanol on the duration of lidocaine narcosis in rats and mice. Lidocaine narcosis was prolonged by a single dose of ethanol of 1.5–3.0 g/kg. Since prolongation of the narcosis due to hypothermia had been precluded by the experimental design, the potentiating effects of ethanol were attributed to one or more of the following possibilities: (a) alteration in absorption or distribution, (b) synergism at the site of action and (c) inhibition of lidocaine metabolism.

Since the areas under the blood radioactivity–time curves (Figure 2) and the $T_{\frac{1}{2}}$ obtained from control and pretreated mice were not significantly different, potentiation by ethanol due to an increased absorption of diazepam could be excluded. The fact that excretion of total radioactivity in the urine and faeces (Figure 3) was not increased by ethanol pretreatment supported this contention.

Examination of bile collected from mice with cannulated bile ducts indicated that biliary excretion was the major route of [^{14}C]-diazepam clearance (Figure 4). The fact that by 12 h, 73.2 ± 3.4% of the dose of ^{14}C could be accounted for in the bile of control mice with cannulated bile ducts (Figure 4) whereas only 50.2 ± 4.7% could be accounted for in the faeces at 48 h (Figure 3) suggests that diazepam metabolites undergo enterohepatic circulation in the mouse, a phenomena reported by Baird & Hailey (1972) for man. Ethanol pretreatment inhibited the 0–12 h cumulative biliary excretion of ^{14}C (Figure 4) and the elevated blood (Figure 2) and tissue (Table 1) concentrations of ^{14}C in ethanol pretreated mice are probably a consequence of this inhibition of biliary excretion.

Ethanol is known to inhibit the drug metabolizing enzyme system (Rubin & Lieber, 1968; Rubin, Gang, Misra & Lieber, 1970; Coldwell, Paul & Thomas, 1973; Mezey, 1976), thus preventing the elimination of lipid soluble material from the body. This possibility was examined. The major metabolite in bile was oxazepam glucuronide (Table 2), and ethanol inhibited the biliary excretion of this metabolite at 2, 4 and 8 hours. In addition the excretion of the radioactive metabolites, which remained at or near the origin after Ketodase treatment of bile, was also decreased by ethanol pretreatment at all time periods examined,

Table 3 Effect of ethanol (3 g/kg, orally) on plasma and brain concentrations of oxazepam and desmethyl diazepam in mice 2 h after administration of [^{14}C]-diazepam (5 mg/kg, orally)

Metabolite	*Pretreatment*	*Plasma (μg/ml)*	*Brain (μg/g)*
Oxazepam	Vehicle	0.54 ± 0.03	1.10 ± 0.03
	Ethanol	0.31 ± 0.03**	0.70 ± 0.03***
Desmethyldiazepam	Vehicle	0.32 ± 0.05	0.56 ± 0.05
	Ethanol	1.46 ± 0.09***	3.12 ± 0.17***

Octanol extracts of plasma and ethylacetate extracts of brain were chromatographed on silica gel HF 250 micron plates using chloroform:*n*-heptane:ethanol (10:10:1). Values are means ± s.e. mean of five observations.

** $P<0.01$; *** $P<0.001$, when compared to vehicle pretreated controls.

suggesting that in addition to inhibition of oxazepam formation, biotransformation of [^{14}C]-diazepam to other polar end products was being inhibited. The increased octanol extractability of ^{14}C from plasmas of ethanol pretreated animals indicated a higher percentage of radioactive lipophilic material in the plasmas of ethanol pretreated mice than in vehicle pretreated controls giving credence to the inhibitory effects of ethanol on diazepam metabolism. The fact that ethanol pretreatment reduced at 2 h the plasma levels of oxazepam (Table 3), but increased desmethyl diazepam concentrations suggests that the inhibition of diazepam metabolism occurred at the metabolic step at which desmethyl diazepam is 3-hydroxylated to give oxazepam. This inhibition of drug metabolism by ethanol has been attributed by Ozols (1976) to a reduction in the transfer of reducing equivalents to the microsomal oxidase system.

Synergism by ethanol with the effect of diazepam on the central nervous system is one possible explanation for the supra-additive or potentiating effects of the ethanol-diazepam combination. However, the major compound found in brain was not diazepam itself, but oxazepam in control mice and desmethyl diazepam in ethanol pretreated mice (Table 3). In mice desmethyl diazepam possessed a lower ED_{50} (4 mg/kg, orally) for the rotarod test than either diazepam (6 mg/kg, orally) or oxazepam (7 mg/kg, orally) (Randall & Kappell, 1973) and brain levels of desmethyl diazepam were higher in ethanol pretreated mice than in controls (Table 3). Therefore the most likely interpretation for the supra-additive effects of diazepam in mice following ethanol administration is that the inhibition of metabolism causes accumulation of a metabolite (desmethyl diazepam) which, in addition to a higher pharmacological activity, also possesses a higher lipid solubility than oxazepam (as indicated by TLC mobilities in system A) resulting in a greater partitioning into lipoid tissue such as the brain.

The mouse metabolizes diazepam in a similar way to man and therefore is a reasonably good animal model for studying drug-diazepam interactions. Extrapolation from mouse to man is not always possible, but data from this study certainly indicate that a diazepam-ethanol interaction is a distinct possibility in man. Even though the therapeutic index of diazepam is large and death as a direct consequence of the diazepam-ethanol combination is not likely to occur with normal therapeutic doses, death and injury as an indirect result of the supra-additive pharmacological effects of this combination can be expected.

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