Effect of Ethanol on Toxicity and Metabolism of Amphetamine in the Mouse

While classical potentiation interactions often occur between ethanol and central nervous system depressants, the effect of ethanol on stimulant action is variable and not well documented[1]. Thus ethanol will antagonize toxic doses of amphetamine in the rabbit[2], alter amphetamine induced behavioural changes in dogs[3] or have a pronounced effect on amphetamine metabolism in the rat[4].

The present study has determined the effect of ethanol on the toxicity (LD_{50}) and urinary metabolite profile of amphetamine in the mouse. Unlike the rat, amphetamine metabolism in the mouse proceeds to a significant extent through the oxidative pathways which are believed to predominate in man[5].

Methods. The acute toxicity of D-amphetamine sulphate in the presence of ethanol (3 g/kg) was determined in male Swiss Webster mice weighing 20 ± 2 g, obtained from the Canadian Communicable Diseases Centre, Ottawa. Individual mice were isolated in opaque plastic cages at 25 °C and the food removed overnight prior to dosing. At least 15 mice were used at each dose level of 10, 30, 50, 70, 90, 110, 130, 150 and 170 mg/kg D-amphetamine sulphate or D-amphetamine sulphate mixed with 15% aqueous ethanol and administered i.p. at 2.0 ml/100 g body wt. Cumulative mortality was determined 2.5 and 24 h after dosing. The LD_{50} values were determined by the method of LITCHFIELD and WILCOXON[6]. The metabolites of amphetamine were examined in urines collected in glass vials following administration of ^{14}C-D-amphetamine sulphate (10 mg/kg) or amphetamine and 2, 3 or 4 g/kg ethanol. Urine collections were made at 6, 12 and 24 h after dosing. Individual urine samples were monitored for total radioactivity, pH and volume and then subjected to descending paper chromatography on strips of Whatman No. 1, at room temperature. The solvent was the organic phase from 3-methylbutan-1-ol; 2-methylbutan-2-ol; water; formic acid (5:5:10:2, by vol)[7]. Metabolites were identified by their Rf values[5] and by co-chromatography with known compounds. After radio-scanning the strips were cut into 1 cm segments and placed in scintillation vials and counted[8]. Metabolites were also visualized by the following sprays: diazotized *p*-nitro aniline for amphetamine and *p*-hydroxy amphetamine[9]; *p*-dimethylaminobenzaldehyde for hippuric acid[10] and 0.04% bromphenol blue for benzoic acid[11].

Results. The dose response curves for amphetamine and amphetamine plus ethanol are shown in the Figure. Ethanol, at 3 g/kg, did not significantly alter the LD_{50} of amphetamine at either time period. The dose response curves exhibit a small mortality increase at lower doses,

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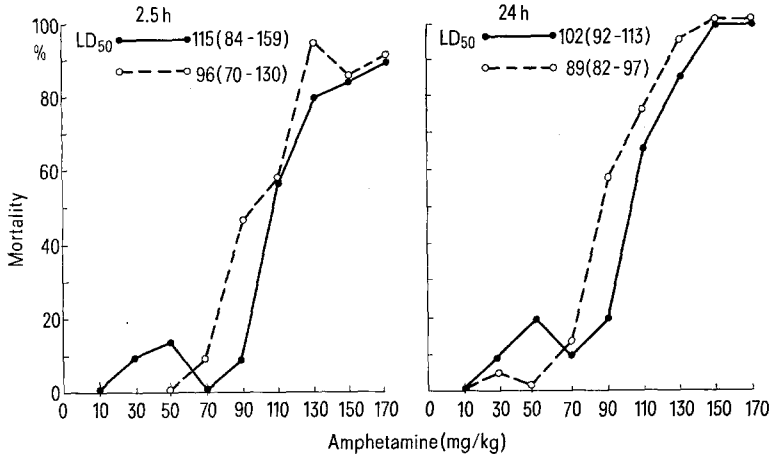

The dose response curves for amphetamine (○—○) and amphetamine + 3 g/kg ethanol (○---○), 2.5 and 24 h after i.p. administration.

Table I. Urinary excretion of amphetamine and its metabolites expressed as percent (± SE) of the total ^{14}C excreted 0–6 h after dosing ($N = 6$)

Dose	*p*-Hydroxy amphetamine glucuronide	*p*-Hydroxy amphetamine	Hippuric acid	Unknown		Amphetamine
				I	II	
Amphetamine (10 mg/kg)	14.4 ± 0.8	3.7 ± 0.6	20.6 ± 2.3	1.5 ± 0.3	15.9 ± 4.9	43.9 ± 3.6
Amphetamine + 2 g/kg ethanol	8.5 ± 0.4	2.2 ± 0.2	19.3 ± 1.8	1.5 ± 0.6	4.2 ± 3.3	64.4 ± 5.1
Amphetamine + 3 g/kg ethanol	4.7 ± 0.3	1.9 ± 0.3	18.9 ± 1.6	1.0 ± 0.5	4.9 ± 2.1	68.6 ± 1.4
Amphetamine + 4 g/kg ethanol	5.9 ± 0.3	2.2 ± 0.3	16.0 ± 1.1	0.4 ± 0.2	3.6 ± 1.1	72.0 ± 2.3

Table II. Mean urine volume (ml) and urinary excretion of amphetamine and total ^{14}C expressed as percent of the dose administered ($N = 6$)

Dose	Total dose excreted (%) and urine volumes (in brackets)			Total dose as unchanged amphetamine (%) (0–6 h)	Total dose excreted in 24 h (%)
	0–6 h	6–12 h	12–24 h		
Amphetamine (10 mg/kg)	70.6 (1.10)	4.5 (1.02)	2.9 (1.61)	31.0	77.9
Amphetamine + 2 g/kg ethanol	62.2 (0.99)	4.8 (0.14) [a]	7.5 (0.59)	39.8	74.6
Amphetamine + 3 g/kg ethanol	55.9 (0.59)	18.7 (0.27)	9.1 (0.90)	38.5	83.7
Amphetamine + 4 g/kg ethanol	55.3 (0.57)	20.4 (0.44)	3.5 (0.67)	39.8	79.2

[a] 2 animals only.

peaking at 50 mg/kg, and a subsequent decline at 70 mg/kg. This phenomenon has been reported by several workers[12,13] but generally with grouped mice, where the mortality at low amphetamine doses is very much greater. In this study ethanol was effective in reducing or eliminating this small initial mortality response. Because of this non-linear response, the LD_{50} calculations were based on the final increase in mortality beginning at 70 mg/kg.

The effects of ethanol on the urinary excretion of amphetamine and its metabolites in the mouse are shown in Table I. Individual metabolites were determined in the 0–6 h urine only. The urine pH was 6.6–7.0. As the ethanol dose increased, the excretion of unchanged amphetamine, expressed as percent of total ^{14}C excreted, increased from 44% to 72%. All other metabolites, with the exception of hippuric acid, decreased as the ethanol dose increased. On the other hand, expressed as a percent of the dose administered (Table II), excretion of total radioactivity decreased from 71% to 55% resulting in only a slight net increase (31% to 39%) in the excretion of amphetamine in the presence of ethanol (Table II). Similarly, at 24 h the excretion of total radioactivity, expressed as percent of the dose administered, was only slightly greater (77.9% to 79.2%) in the presence of 4 g/kg ethanol.

Discussion. The lack of effect of ethanol on the acute toxicity of amphetamine would suggest that the alcohol is not significantly altering interaction of the amine with its receptor site. A decrease in the initial mortality phase at 50 mg/kg was noted, but it is difficult to explain this action since the phenomenon is probably seen as a residual effect of grouping stress.

The inhibitory effect of ethanol on metabolism is striking if one considers the percentage increase in unchanged amphetamine appearing in the 0–6 h urines (Table I). In terms of percent of amphetamine administered, the inhibitory effect of ethanol is not as marked, increasing unchanged amphetamine in the urine from 31.0% to 39.8% (Table II). In this context, 2 g/kg ethanol was just as effective as 4 g/kg in increasing 0–6 h urinary output of unchanged amphetamine.

The major effects of ethanol appear to be a decrease in urine volume, noted at higher levels of ethanol, coupled with an inhibition of metabolism or excretion of metabolites in the 0–6 h period. This is consistent with the large increase of urinary radioactivity (20%) in the 6–12 h urines at 4 g/kg ethanol compared to the 4% output with amphetamine alone. Some effect on metabolism would be expected since the metabolism of ethanol itself would change the NAD/NADH ratio of the liver. These effects however do not have a significant effect on toxicity since the LD_{50} value at 2.5 h is little different from that at 24 h.

Summary. Ethanol, 3 g/kg i.p., did not significantly alter the acute toxicity of amphetamine in the mouse. However, the urinary metabolite pattern was changed, suggesting that ethanol suppressed metabolism of the stimulant during the initial 6 h period. After 24 h, the mouse metabolized the same fraction of a given dose of amphetamine, whether it was given as amphetamine alone or amphetamine mixed with 2, 3 or 4 g/kg ethanol.

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In vivo and in vitro Studies on Irreversible Binding of Halothane Metabolites to Proteins

The halothane hepatitis is a very rare but extremly serious incident. The reason for its development has not been elucidated. Its course and symptoms point to an allergic process[1–3]. However, the halothane molecule does not posess the chemical properties essential for reacting as hapten with proteins in order to form an antigenic molecule[4–6]. This seems to be the reason why many attempts have failed to elicit with halothane a transformation of lymphocytes from patients having survived a halothane hepatitis[7,8]. Efforts to sensitize rabbits against trifluoracetic acid[9], the main metabolite of halothane, did not present convincing results. The chemical coupling

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