Correlation of (+)-amphetamine lethality and hepatic metabolism

Several investigators have observed that the pattern of lethality in mice treated with (+)-amphetamine shows a rise then a fall followed by a rise as the dose of amphetamine increases (Höhn & Lasagna, 1960; Askew, 1962; George & Wolf, 1966; Gardocki, Schuler & Goldstein, 1966; Stolk & Rech, 1968). To date, the pharmacological interpretations of the characteristic "triphasic" shape of the lethality curve for (+)-amphetamine have been largely speculative. We have sought to determine whether a relation exists between the dose-dependent pattern of hepatic (+)-amphetamine metabolism and the triphasic lethality curve.

Lethality was determined 3 h after intraperitoneal injection into isolated male CF-1 mice of various doses of (+)-amphetamine according to George & Wolf (1966). Using the Litchfield-Wilcoxon (1949) method of statistical analysis, the two LD50 values calculated for (+)-amphetamine were 19·0 mg kg^{-1} (13·7–26·4 mg kg^{-1}) and 77 mg kg^{-1} (70·3–84·4 mg kg^{-1}).

The metabolism of (+)-amphetamine was investigated in liver microsomes isolated from CF-1 mice. Incubation mixtures contained substrate, 8 mg microsomal protein, an NADPH-generating system (2·4 μmol $NADP^+$, 15 mg glucose-6-phosphate, 2 E.U. glucose-6-phosphate dehydrogenase and 15 μmol $MgCl_2$) and 150 μmol tris-HCl buffer, pH 7·4, containing 1·15% KCl, in a final volume of 3·0 ml. Preparation of microsomes and assay of amphetamine disappearance was as described by Gerald & Feller (1970) and Axelrod (1954). A triphasic pattern of hepatic microsomal (+)-amphetamine metabolism was observed. The rate of (+)-amphetamine disappearance at 0·67 mM (3·2 $\pm$ 0·6 nmol mg^{-1} protein in 30 min) was significantly lower ($P < 0.05$) than the metabolic rates obtained at either 0·33 or 1·0 mM (10·5 $\pm$ 1·9 and 16·0 $\pm$ 2·4 nmol mg^{-1} protein in 30 min respectively) (n = 3 or 4).

In an attempt to modify the pattern of (+)-amphetamine metabolism in mouse liver microsomes, groups of mice were pretreated with the known hepatic enzyme inducers, spironolactone (Feller & Gerald, 1971) and phenobarbitone (Conney, 1967). The results (Table 1) show that pretreatment with spironolactone did not alter the rate of (+)-amphetamine disappearance at 0·33 mM, but completely abolished the

Table 1. *Effect of pretreatment with spironolactone and phenobarbitone on the metabolism of (+)-amphetamine in liver microsomes from CF-1 mice.* Experiment I: Mice were given spironolactone (100 mg kg^{-1}) or corn oil i.p. twice daily for 3 days. Experiment II: Mice were given phenobarbitone (50 mg kg^{-1}) or saline i.p. twice daily for 3 days. Metabolic experiments were initiated 16 h after the last dose. Incubation mixtures contained the components described in the previous experiment. Values represent the mean $\pm$ s.e. of 4–6 determinations.

Treatment	Amphetamine disappearance nmol mg^{-1} in 30 min at substrate concentrations of 0·33	0·67mM
Experiment I: Corn oil	9·9 $\pm$ 1·8	2·5 $\pm$ 1·3
Spironolactone	9·4 $\pm$ 2·0	13·5 $\pm$ 1·2*
Experiment II: Saline	14·6 $\pm$ 2·5	4·6 $\pm$ 1·7
Phenobarbitone	15·1 $\pm$ 0·3	4·5 $\pm$ 1·0

* Significantly different ($P < 0.05$) from the control group at the same substrate concentration.

reduction in microsomal metabolism at 0·67 mM in the control preparation. In contrast, the prior administration of phenobarbitone did not modify the rates of amphetamine disappearance at either dose level when compared with the rates obtained in control microsomes. In agreement with the latter observation, Prabhu (1972) reported that the toxicity of (+)-amphetamine in mice remained unchanged by chronic administration of phenobarbitone. Dr. Wolf (personal communication) tells us that spironolactone pretreatment in CF-1 mice, reduced both acute and aggregated (+)-amphetamine lethality (100 mg kg^{-1}, i.p.), but that only the latter parameter differed significantly from the non drug-pretreated animals.

Thus, while the data show that qualitatively similar dose-dependent patterns are observed with hepatic biotransformation and lethality of (+)-amphetamine in CF-1 mice, the interrelation between these two parameters is less clear. For example, we have observed that while (−)-amphetamine exhibited a triphasic response in hepatic microsomal metabolism, this isomer gave only a single LD50 value. These findings suggest the need for additional *in vivo* and *in vitro* experiments to clarify the relation between amphetamine biotransformation and lethality.

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