KINETICS OF N,N-DIMETHYL TYRAMINE (HORDENINE) DEMETHYLATION BY GUINEA-PIG LIVER MICROSOMES

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The sympathomimetic amine hordenine occurs in food products containing the red alga Gigartina stellata (Barwell and Blunden, 1981). It could be pharmacologically inactivated by N-demethylation and deamination. A previous study, using a single non-saturating concentration, indicated that hordenine may not be metabolised via microsomal N-demethylation (Barwell et al 1984). In order to more fully assess the role of N-demethylation a kinetic investigation was carried out, in which the Michaelis constant (Km) and maximum velocity (Vmax) for hordenine were determined, in comparison with some other tertiary amines: N,N-dimethyl aniline (DMA) and N,N-dimethyl phenethylamine (DMP)

Female guinea pigs (400-500g) were used. Microsomal enzymes were induced with sodium phenobarbitone administered i.p. at 25 mg per kg for 4 days. Microsomes were isolated by differential centrifugation of homogenates prepared in 0.3M sucrose. Demethylation was determined at pH 7.4 and 37°C by measuring formaldehyde production using Nash reagent. DMP was synthesised for the investigation.

Uninduced-microsomes demethylated DMA with a Vmax of 130 nmol per mg protein per hour but the rate with hordenine was too low to permit accurate determination of kinetic constants. Induced-microsomes exhibited a three-fold higher activity and were used for kinetic experiments. For each tertiary amine, plots of velocity (v) against substrate concentration (s) were apparently hyperbolic and Hanes plots (s/v against s) were linear. Thus, under the experimental conditions, N-demethylation obeyed normal Michaelis-Menten kinetics. Table 1 contains Km and Vmax values for DMA, DMP and hordenine. Both the affinity and Vmax for these substrates decreased in the order DMA > DMP > hordenine. The apparent Km for hordenine was twelve times lower than for DMP. This marked effect is attributable to the lower lipid solubility of hordenine, due to its para hydroxyl group.

Table 1. Kinetic parameters of microsomal N-demethylation

Substrate	Km (mM)	Vmax (nmol.(mg protein)$^{-1}$. h^{-1})
N,N-dimethyl aniline	0.95	404
N,N-dimethyl phenethylamine	3.71	103
Hordenine	43.50	53

The kinetic parameters in table 1 were obtained with induced-microsomes which exhibited a three-fold higher capacity for DMA demethylation than uninduced-microsomes. Therefore, it may be expected that with uninduced-microsomes, the Vmax for hordenine would be approximately 18 nmol per mg protein per hour. This low capacity, together with the very low affinity, shows that hordenine is a poor substrate for microsomal N-demethylation. Other microsomal transformations such as N-oxidation (Gorrod and Patterson 1983) and meta hydroxylation followed by O-methylation (Daly et al 1965) would occur at lower rates. Thus the pharmacological inactivation of hordenine should depend upon monoamine oxidase.

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