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Deuterium isotope effect on the enzymatic oxidation of dopamine and serotonin

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Monoamine oxidase (EC 1.4.3.4, MAO) inactivates various amines. The reaction involves the cleavage of an α-hydrogen from the side-chain carbon atom. It has been observed that substitution of this α-hydrogen with deuterium in *p*-tyramine results in a profound reduction in the rate of oxidation [1, 2]. This deuterium isotope effect was found to be related to hydroxyl substitution on the phenyl ring with respect to *p*-tyramine, *m*-tyramine and β-phenylethylamine [1]. We have now investigated further the deuterium isotope effect in the oxidative deamination of the putative neurotransmitters, dopamine and serotonin (5-HT).

Rat liver mitochondrial MAO was used in this study. The mitochondrial fractions were obtained by differential centrifugation, and the mitochondrial membrane fragments were prepared by lysing the mitochondria in chilled distilled water and then centrifuging at 105,000 *g* for 30 min as previously described [1]. The membrane preparations were then washed by suspension in chilled distilled water and centrifuged. MAO activity was measured using a high performance liquid chromatographic (HPLC) method based on the estimation of the disappearance of substrates [3]. The enzyme was incubated in 1×10^{-4}M non-deuterated or $\alpha\alpha$-d_2, $\beta\beta$-d_2, and $\alpha\alpha\beta\beta$-d_4 deuterated dopamine or serotonin in 0.05 M phosphate buffer (pH 7.5). The reactions were stopped by addition of 1% perchloroacetic acid containing isoproterenol or *N*-methyldopamine as internal standard for the HPLC system. The system consisted of a Waters (Melfort, MA) model 6000 solvent delivery pump, a Waters model UK injector, an Altex (Arlington Heights, IL) Ultrasphere C_{18} ion pairing column, a Bioanalytical System LC-2A electrochemical detector (West Lafayette, IN) with carbon paste electrode, and a Fisher Recordall series recorder. A base solvent of 0.1 N HNO_3 titrated to pH 2.7 with concentrated NaOH and containing 20 mg/l EDTA was modified by adding 2% methanol for dopamine analysis and 10% methanol for serotonin analysis. A rate of 1 ml/min was maintained for dopamine and 0.9 ml for serotonin. The internal standards were *N*-methyldopamine for dopamine measurements and isoproterenol for serotonin analysis. Calibration curves were prepared daily prior to the analysis. An electrode potential of 0.72 V with respect to Ag/AgCl reference electrode was used. A 1–5 μl injection and a sensitivity range of 10–20 nano-amps/V gave a good signal for the measurement of peak heights.

Dopamine-$\alpha\alpha$-d_2 was prepared by reduction of 3,4-dimethoxyphenylacetonitrile with lithium aluminum deuteride and, then, demethylation in glacial acetic acid and hydrogen bromide. Dopamine-$\beta\beta$-d_2 was prepared by refluxing 3,4-dimethoxyphenylacetonitrile in ethanol-OD, -D_2O, -NaOD and KCN, followed by reduction with lithium aluminium hydride and demethylation. The deuterated dopamine analogues were then isolated and purified by recrystallization of the hydrochloride from ethanol-ether. 5-Hydroxytryptamine-$\alpha\alpha$-d_2 was prepared from 5-benzyloxyindole-3-acetonitrile. The nitrile ester was reduced with lithium aluminium deuteride and hydrogenated over palladium on charcoal to remove the benzyl

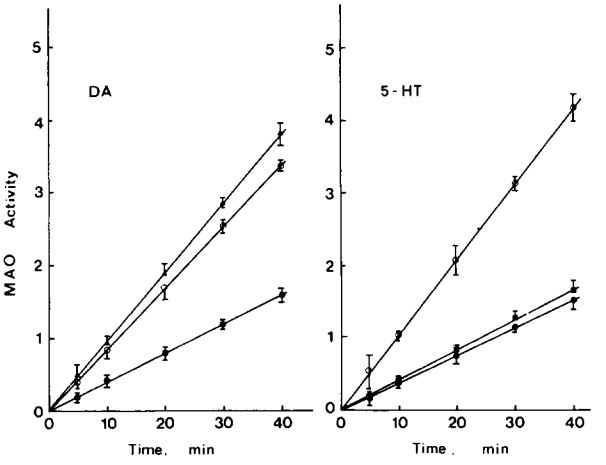

Fig. 1. Initial velocities of the deamination of different deuterated analogues of dopamine and serotonin. Enzyme activity [nmoles · (mg protein)$^{-1}$ · min^{-1}] was measured using the HPLC method with $\alpha\alpha$-d_2 (●——●), $\beta\beta$-d_2 (▲——▲), $\alpha\alpha\beta\beta$-d_4 (■——■), and nondeuterated (○——○) amines as substrate. The substrate concentrations used were 1×10^{-4} M. Each value is the means ± S.E.M. of triplicate determinations.

Table 1. Comparison of the apparent V_{max} and Michaelis–Menten constants for MAO in the enzymatic deamination of dopamine and serotonin labeled in the α-position with deuterium*

	Dopamine		Serotonin	
	V_{max}	K_m	V_{max}	K_m
N n-deuterated	7.14 ± 0.81	4.3 ± 0.1	6.06 ± 0.50	1.46 ± 0.09
$\alpha\alpha$-d_2-labeled	4.43 ± 0.24†	6.1 ± 0.70‡	2.47 ± 0.12†	2.23 ± 0.03†

* V_{max} [maximum velocities, nmoles · (mg protein)$^{-1}$ · min^{-1}] and K_m [Michaelis–Menten constant, 1×10^{-4} M] values were obtained by Lineweaver–Burk double-reciprocal plots. Experimental data were based on least squares analysis assuming equal variance for the velocities.

† Significantly different from the control, $P < 0.001$.

‡ Significantly different from the control, $P < 0.05$.

Table 2. Deuterium isotope effects on the enzymatic deamination of $\alpha\alpha$-d_2-labeled dopamine and serotonin

Substrate	V_H/V_D	$(V/K_m)_H/(V/K_m)_D$
Dopamine	1.61	2.28
Serotonin	2.45	3.75

group; then it was isolated and purified as its creatinine salt. 5-Hydroxytryptamine-$\alpha\alpha\beta\beta$-d_4 was prepared by refluxing 5-benzyloxyindole-3-acetonitrile, in ethanol-OD, D_2O and NaCN, reduction with lithium aluminum deuteride and hydrogenation over palladium on charcoal.

The extent of deuteration was assessed mass-spectrometrically utilising the dansyl derivatives of the unsubstituted and deuterated amines with an A.E.I. MS-902S high resolution mass spectrometer [1]. It was found that the extent of deuteration was quite high in all cases (> 95%).

The enzymatic deamination of dopamine and serotinin and their deuterated analogues was determined. As can be seen from Fig. 1, the initial rates of oxidative deamination of the $\alpha\alpha$-d_2-dopamine and $\alpha\alpha$-d_2-5-HT were much reduced in comparison with those of the non-deuterated amines by the rat liver mitochondrial MAO. A slight enhancement of MAO activity with respect to $\beta\beta$-d_2-dopamine and a reduction with respect to $\alpha\alpha\beta\beta$-d_4-5-HT, as was the case for the trace amines [1], were also observed.

The kinetic parameters K_m and V_{max} for DA and 5-HT as derived from Lineweaver–Burk plots are summarized in Table 1. A decrease in V_{max} and an increase of K_m were observed with respect to the oxidation of both $\alpha\alpha$-d_2-dopamine and $\alpha\alpha$-d_2-serotonin. The isotope effects of the $\alpha\alpha$-d_2 substitution, expressed as V_H/V_D and $(V/K_m)_H/(V/K_m)_D$ are shown in Table 2. The magnitude of these deuterium isotope effects, as compared with our previous observation of the trace amines [1], was in the order: serotonin > p-tyramine > m-tyramine > β-phenylethylamine > dopamine. The aromatic structures of these amines appear to have affected the transition state and varied the isotope effect.

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Liver cell plasma membrane lipids in manganese–bilirubin-induced intrahepatic cholestasis

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In the rat, administration of manganese followed by injection of bilirubin results in a severe reduction in bile flow; this cholestasis is reversible, varies according to the dose of bilirubin administered, and can be prevented by the administration of sulfobromophthalein [1–6]. Its pathogenesis is not yet known. In a previous study, de Lamirande *et al.* [7] observed that alteration of the enzymic activity of liver cell plasma membranes did not appear to be the cause of cholestasis. However, they reported that a marked shift in the recovery of protein in isolated liver plasma