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COMPARISON OF THE BRAIN LEVELS OF *N*,*N*-DIMETHYLTRYPTAMINE AND $\alpha,\alpha,\beta,\beta$-TETRADEUTERO-*N*,*N*-DIMETHYLTRYPTAMINE FOLLOWING INTRAPERITONEAL INJECTION

THE *IN VIVO* KINETIC ISOTOPE EFFECT*

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Abstract—A comparison of the brain levels (μg/g wet weight of tissue) of the hallucinogen *N*,*N*-dimethyltryptamine (DMT) and its deuterated analog $\alpha,\alpha,\beta,\beta$-tetradeutero-DMT ($D_4$DMT) as a function of time and dose is reported. It was observed that the presence of deuterium in the α- and β-positions of the ethylamine side-chain led to a potentiation of the level of DMT in brain. Strikingly different dynamics of uptake and clearance were also noted. We propose that these results are due to a primary kinetic isotope effect, illustrating the importance of the α-position in the metabolism of DMT.

The major metabolite of the hallucinogen *N*,*N*-dimethyltryptamine (DMT) has been identified both *in vitro* and *in vivo* as indoleacetic acid (IAA) (for a review, see Ref. 1). It has been debated whether IAA is predominantly formed from initial direct deamination of DMT by monoamine oxidase (MAO, EC 1.4.3.4) or by the action of this enzyme on the intermediary metabolites *N*-methyltryptamine (NMT) and DMT-*N*-oxide (DMT-NO) [2, 3]. The belief that DMT is significantly metabolized via direct deamination by MAO has been supported by the observation that pretreatment of test animals with a MAO inhibitor (pargyline, iproniazid, etc.) potentiates the behaviour-disrupting effects, tissue levels, and other pharmacological events produced by DMT [3–7]. However, this is in contradiction to studies which have shown that DMT is not appreciably metabolized by purified preparations of MAO [8–11] as well as other studies which have reported that tertiary amines, in general, are poor substrates for this enzyme [9, 12].

In this report, we present data on the effect that substitution of deuterium (D) for hydrogen (H) at the α- and β-positions of the ethylamine side-chain has on the brain levels of DMT, i.e. a measurement of the kinetic isotope effect *in vivo* (for a review, see Ref. 13). The mechanism for the deamination of indole-ethylamines is proposed to involve the breaking of the α-C-H bond via oxidation by MAO. Thus, the greater bond energy of a C-D bond in this position would be expected to produce a decrease in the rate of metabolism of DMT by direct deamination [13] which may be translated into higher tissue levels of DMT. Since the mechanisms for the *N*-demethylation and *N*-oxidation of DMT are not proposed to involve the α-C position [2], $\alpha,\alpha,\beta,\beta$-tetradeutero-DMT ($D_4$DMT) was postulated to be a useful tool for the examination of the importance of the direct deamination of DMT in its overall metabolism and deactivation, without requiring the prior inhibition of MAO.

MATERIALS AND METHODS

Materials. The D_4DMT and DMT were provided by Professor Fred Benington and Dr. Richard Morin of this laboratory. Heptafluorobutyrylimidazole (HBFI) was obtained from the Pierce Chemical Co., Rockland, IL. All other reagents were obtained from commercial sources and were of the highest available purity.

Subjects. Male Long–Evans rats weighing between 250 and 300 g were used in the study. The animals were individually housed and fed and watered *ad lib*.

Drug treatment. Sixty rats were divided into three groups of twenty rats each and injected intraperitoneally (i.p.) with D_4DMT (dissolved in isotonic saline adjusted to a pH of 6.0 with 0.1 N HCl), at three different dose levels: 2.5 mg/kg, 5.0 mg/kg, or 10.0 mg/kg. Four rats from each group were decapitated at 5, 10, 20, 40 and 80 min postinjection. The brains were rapidly excised, frozen in liquid N_2 and stored at −76° until analyzed. Similarly, one group of twenty rats was injected with 5.0 mg/kg of DMT and killed as described above to provide for a comparison of D_4DMT and DMT brain levels.

Tissue extraction and gas chromatographic/mass spectrometric (GC/MS) analysis. The individual brain samples were weighed and added to tubes

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containing 2.0 ml of glass-distilled H_2O, 0.5 ml of 70% $HClO_4$, and 1000 ng/g brain tissue of internal standard (DMT for the D_4DMT analyses and D_4DMT for the DMT analyses). The samples were thoroughly homogenized (Polytron) and were then extracted and derivatized according to the methods previously described by this laboratory [14, 15]

The analyses of the HFB derivatives of DMT and D_4DMT were conducted using a Hewlett Packard 5985A GC/MS equipped with a data analysis system. Gas chromatography was conducted on a 25 meter by 0.2 mm inside diameter SP2250 fused silica capillary column (Supelco). The injection port and transfer line temperatures were maintained at 270°. A temperature program beginning at 50°, holding for 1 min and then increasing to 270° at 30°/min, was used. The GC analyses were made in the splitless mode, and the purge function was activated 1 min after injection. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The effluent from the column was led directly into the mass spectrometer, which was operated in the electron-impact mode at an ionizing energy of 70 eV. The analyses were conducted in the selected ion monitoring mode (SIM) at an electron multiplier setting of 3000 V. The mass spectrometer was tuned daily using perfluorotributylamine as the calibration standard.

The mass spectra and selected ions for the identification and quantification of DMT and D_4DMT have been reported previously by this laboratory [2, 16]. The ions (m/z) chosen to monitor for DMT were 58.2 and 129.1. The ions monitored for D_4DMT were 60.2 and 131.1. Pure standards of each compound were analyzed separately in the SIM mode to determine the percent interference between the ions. Using this method, D_4DMT was found to have a 99.8% isotopic purity with respect to the ions for DMT, and DMT was found to have a 99.9% isotopic purity with regard to the ions for D_4DMT.

Linearity of recovery. A standard curve for the determination of the linearity of recovery of DMT and D_4DMT from rat brain was obtained by adding increasing amounts of D_4DMT (10, 20, 40, 50, 100, 1000 and 2000 ng) and a constant amount of DMT (2000 ng) to pooled rat brain homogenates. These samples were treated and analyzed as described above.

Quantification of DMT and D_4DMT. Quantification of DMT and D_4DMT was accomplished by comparing ion ratios (58.2/60.2 or 60.2/58.2), correcting for interference, and then multiplying this value times the amount of internal standard added. This value was divided by the brain weight and expressed as μg of DMT or D_4DMT per g wet weight of brain.

RESULTS

Recovery of D_4DMT/DMT from rat brain was linear ($r = 0.98$) over a wide range of concentrations (Fig. 1). The correlation between ion ratios for the individual compounds (129.1/58.2 for DMT compared to 131.1/60.2 for D_4DMT) was near unity (1.01 ± 0.13) and supported the identification of the peaks as DMT or D_4DMT. A representative chromatogram for the analysis of brain samples for DMT and D_4DMT is shown in Fig. 2. The results of the GC/MS analyses of rat brain from animals injected with 5.0 mg DMT/kg and those injected with 2.5, 5.0 or 10.0 mg D_4DMT/kg are presented in Table 1. These values are compared to those reported previously for the levels of DMT in brain over time following an i.p. injection [17, 18]. The brain level of DMT obtained in the present study is in general

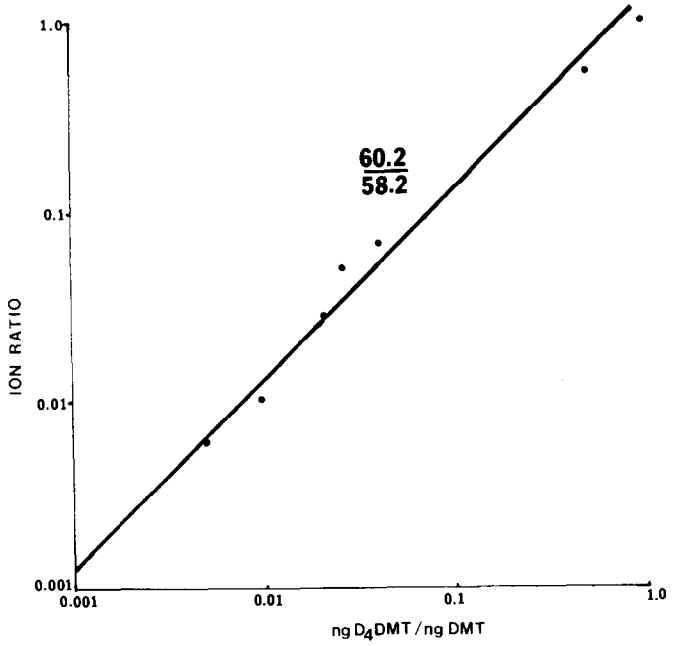

Fig. 1. A log/log scale plot of the recovery of increasing amounts of D_4DMT (m/z = 60.2) and a constant amount of DMT (2000 μg, m/z = 58.2) from rat brain. $r = 0.98$.

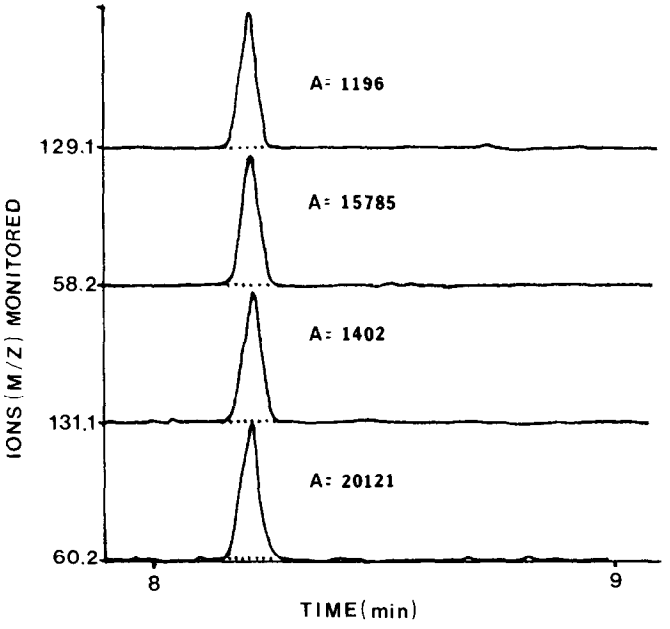

Fig. 2. A representative chromatogram for the SIM analysis of D_4DMT and/or DMT in rat brain. The example shown is from the analysis of brain tissue obtained from a rat injected with 5.0 mg DMT/kg followed by sacrifice at 20 min post-injection. A = area counts.

agreement with that reported by Cohen and Vogel [17] for 5.0 mg DMT/kg administered i.p. (Table 1). The higher levels observed here may be due to differences in the strain of animal used and/or to the possibly greater recovery, sensitivity, and selectivity afforded by the use of internal standards and GC/MS analysis for DMT versus its analysis by spectrofluorometric techniques [6, 17, 18].

The brain level of D_4DMT attained following an i.p. injection of 5.0 mg D_4DMT/kg was significantly different ($P \leq 0.05$) from that observed for DMT at the 5- and 40-min time intervals. The level of D_4DMT in brain observed after injections of 2.5 mg D_4DMT/kg was also significantly different from the brain level of DMT (5.0 mg/kg) at the 5-min ($P \leq 0.01$) and 40-min ($P \leq 0.05$) time intervals (Student's *t*-test). The failure to reach significance at the other time points was apparently due to the rather large standard deviations within groups, the relatively small *N* (of 3 or 4 for each point), and the quite different dynamics of uptake and clearance observed between D_4DMT and DMT (Table 1). The apparent peaking of D_4DMT concentration at 5 and 40 min did not allow for the calculation and comparison of half-lives. However, D_4DMT was detected in brain tissues for up to 80 min at all dose levels tested, whereas DMT at 5.0 mg/kg was not detected in brain after the 40-min time interval.

DISCUSSION

The kinetic isotope effect. Inhibition of MAO has been shown to potentiate the tissue levels and extend the half-life of DMT [4–7]. The present study illustrates that the substitution of D for H in the α- and β-positions of the ethylamine side-chain of DMT produces a similar effect. Although the possible involvement of the D in the α- or β-positions alone has not been examined here, recent studies have shown that the presence of D in the β-position of aromatic ethylamines actually produces a slight enhancement in the rate of deamination [19]. Thus, the potentiation of DMT brain levels observed in the present study may be directly attributed to the involvement of the α-position in the metabolism of DMT. This would be indicative of a metabolic pathway requiring the cleavage of the C—H bond in the α-position, leading to deamination. Substitution of D for H, given the greater bond energy for C—D, would thus be expected to slow the rate of deamination [13, 19]. This kinetic isotope effect may then be translated into higher tissue levels and an increased time of detectability for DMT in brain. This metabolic sequence most likely involves MAO and is supported by studies in which a potentiation of the effects of DMT following MAO inhibition was observed, even though the use of MAO inhibitors may have a significant effect on other metabolic processes [2]. One may conclude from these results that DMT is significantly metabolized by direct deamination and that a substantial portion of the IAA formed during the metabolism of DMT arises from this pathway.

Another phenomenon that was consistently observed in the present study was the rather different dynamics of uptake and clearance exhibited between DMT and D_4DMT (Table 1). This may serve as an indication that the protons of the α- and/or β-position of DMT, perhaps through hydrogen bonding, are involved in mechanisms for the transport and clearance of DMT, in addition to their involvement in its metabolism. However, the observed patterns may also have been due to altered metabolism and reuptake of D_4DMT in the periphery.

Table 1. Brain levels of DMT and D_4DMT as a function of dose and time

Drug	Dose (mg/kg)	Drug [μg/g brain tissue (± S.D.)] Time (min) 5	10	15	20	25	30	40	75	80
DMT	5.0	1.34 ± 0.78	1.50 ± 0.78		0.79 ± 0.69			0.47 ± 0.31		0.0
DMT*	5.0	0.9 ± 0.5	1.0 ± 0.2	1.8 ± 1.2			0.0			
										0.041 ±
D_4DMT	5.0	4.14 ± 1.56	2.30 ± 1.20		1.81 ± 0.63			1.88 ± 0.88		0.007
										0.038 ±
D_4DMT	2.5	5.10 ± 0.67	0.72 ± 0.18		0.78 ± 0.01			1.04 ± 0.02		0.001
DMT†	3.2	1.59 ± 0.36	1.53 ± 0.17	0.93 ± 0.14	0.49 ± 0.12	0.38 ± 0.15	0.0			
D_4DMT	10.0	1.49 ± 0.87	8.46 ± 1.46		14.49 ± 4.05			8.18 ± 0.54		1.04 ± 0.37
DMT†	10.0	4.08 ± 0.30	6.42 ± 0.65	5.06 ± 0.64	3.28 ± 0.37		2.62 ± 0.30		1.76 ± 0.16	0.44 ± 0.05

* Values taken from Ref. 17.
† Values taken from Ref. 18.

Preliminary data obtained from a comparison of the behaviour-disrupting effects of equal doses of DMT and D_4DMT have indicated that D_4DMT has a greater potency than DMT. In agreement with the results presented here, Beaton *et al.** have observed a shorter time to outset, a prolongation of disruption, and a potentiation of the behaviour-disrupting effects of D_4DMT when compared to the effects of equal doses of DMT.

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