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METABOLISM OF THE HALLUCINOGEN *N,N*-DIMETHYLTRYPTAMINE IN RAT BRAIN HOMOGENATES*

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Abstract—The metabolism of the hallucinogen *N,N*-dimethyltryptamine (DMT) in whole rat brain homogenate is reported. Studies were conducted using tritiated DMT and DMT-*N*-oxide (DMT-NO), and metabolites were identified and quantified using thin-layer chromatography and liquid scintillation counting techniques. Metabolite confirmation was obtained by incubation of $\alpha,\alpha,\beta,\beta$-tetradeutero-DMT (DDMT) with whole brain homogenate followed by combined gas chromatographic/mass spectrometric analyses. The metabolites of DMT were identified as indoleacetic acid (IAA), DMT-NO, *N*-methyltryptamine (NMT), 2-methyl-1,2,3,4-tetrahydro-β-carboline (2-MTHBC), tryptamine (TA) and 1,2,3,4-tetrahydro-β-carboline (THBC). DMT-NO was metabolized to give DMT, NMT, IAA and 2-MTHBC. Formation of these metabolites from DMT-NO was stimulated by anaerobic incubation. Mechanisms for the formation of β-carbolines from DMT and DMT-NO are discussed. The effects of the monamine oxidase inhibitor iproniazid phosphate on DMT metabolism were also studied. Iproniazid inhibited the formation of IAA from DMT by 83 per cent. However, the formation of NMT and DMT-NO was inhibited by 90 per cent under these conditions. Thus, the reported extension of half-life and potentiation of DMT behavioral effects by iproniazid may be due to inhibition of NMT and DMT-NO formation rather than inhibition of monoamine oxidase. A cyclic pathway for the synthesis and metabolism of DMT in brain tissue is proposed.

In 1952, Osmond and Smythies [1] proposed the *in vivo* synthesis of mescaline-like hallucinogens as a factor in the etiology of schizophrenia. Following the discovery of enzymes which catalyze the *O*- and *N*-methylation of catecholamines as well as indoleamines [2], this endogenous hallucinogen hypothesis was extended to include the *in vivo* formation of indole-containing hallucinogens, such as *N,N*-dimethyltryptamine (DMT, I, Fig. 1) [3, 4]. Indole-*N*-methyltransferases (INMT), utilizing *S*-adenosylmethionine (SAM) as their methyl source, have since been identified in human lung, brain, blood and cerebrospinal fluid [5], and subsequent analyses have led to the identification of DMT in man [6–14]. It is now apparent that hallucinogenic compounds such

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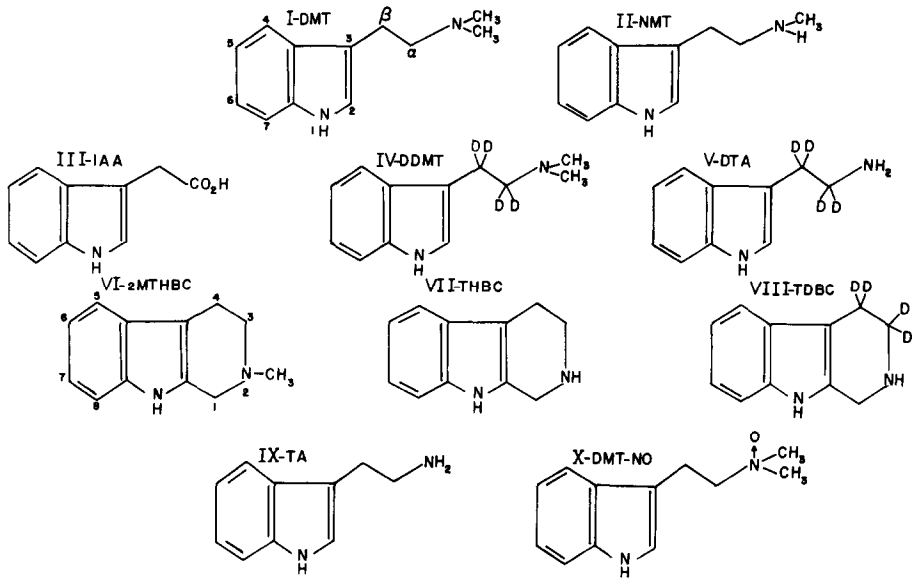

Fig. 1. Compounds referred to in this study.

as DMT are, in fact, normal products of human metabolism. Thus, interest in DMT has been stimulated by its pharmacological properties as a hallucinogenic agent. It is rather unique among the hallucinogens in that an intramuscular injection takes effect in 1–5 min and produces an intense hallucinogenic state lasting only 30–120 min [15–18]. Furthermore, several studies have observed that tolerance to the behavior-disrupting effects of DMT does not develop [19–21]. One study did obtain tolerance in rats by administering DMT every 2 hr for 21 days [22]. The rapid metabolism of DMT has been offered as an explanation for both its short lasting effects and for its failure to elicit tolerance [18]. DMT is rapidly cleared from the blood stream (15–30 min) and metabolized (20–120 min) in both man and other mammals [12, 15, 18, 23–27]. Several investigators have concluded that a correlation between either the presence and/or levels of DMT and schizophrenia has yet to be demonstrated because the compound is rapidly metabolized [25, 26]. Given the rapid metabolism of this compound, measurement of DMT alone *in vivo* may not give an accurate estimation of pre-existing levels or the overall rate of synthesis.

It has been stated that the major routes of DMT metabolism *in vivo* are 6-hydroxylation, *N*-demethylation [15] and oxidation by monoamine oxidase (EC 1.4.3.4, MAO) [15, 27–30]. While several studies have demonstrated that the behavioral effects and tissue levels of DMT in rats are potentiated by pretreatment with the MAO inhibitor (MAOI) iproniazid [27–29], other studies have shown that DMT is not appreciably metabolized by purified preparations of MAO [31, 32] and is, in fact, itself an MAOI [33–35]. It has been proposed that tetrahydro-β-carbolines (THBCs) may also be formed as metabolites of DMT [36, 37]. However, the formation of β-carbolines from DMT, either *in vitro* or *in vivo*, has not been demonstrated.

Although a pathway for the metabolism of DMT has been suggested [24], the actual pathways and the relative importance of the enzymes and metabolites involved in the metabolism of this endogenous hallucinogen are still unclear. This is due mainly to the fact that the recovery of DMT and its related metabolites in *in vivo* studies has been quite low (2.7 to 33 per cent) [12, 13, 15, 23–26] and the *in vitro* metabolism of DMT has been studied in liver homogenates only qualitatively [24, 36]. Neither the *in vivo* nor the *in vitro* studies have to date been concerned with the quantification of relative abundance of the individual metabolites formed. The low recovery of metabolites in the *in vivo* studies has precluded obtaining such information. This lack of information is especially true in the case of DMT metabolism in mammalian brain. To date, only one study has been conducted on the *in vitro* metabolism of DMT in brain tissue [38]. However, this study was also qualitative in nature.

The aim of the present study, therefore, was to identify and quantitate the metabolites of DMT in whole rat brain homogenate. The effects of the MAOI iproniazid on the metabolism of DMT were also observed. From the data obtained in this study, a pathway for the metabolism of DMT in brain tissue is proposed.

MATERIALS AND METHODS

Authentic samples of DMT, *N*-methyltryptamine (NMT, II), indoleacetic acid (IAA, III), and $\alpha,\alpha,\beta,\beta$-tetradeutero-DMT (DDMT, IV) were provided by Professors Fred Benington and Richard Morin of this laboratory. A sample of $\alpha,\alpha,\beta,\beta$-tetradeutero-tryptamine (DTA, V) was purchased from Merck, Sharpe & Dohme Isotopes, Montreal, Canada. Samples of 2-methyl-1,2,3,4-tetrahydro-β-carboline (2-MTHBC, VI), 1,2,3,4-tetrahydro-β-carboline (THBC, VII) and 1,2-dihydro-3,3,4,4-tetradeutero-β-carboline (TDBC, VIII) were prepared from NMT, tryptamine (TA, IX) and DTA, respectively, by the method of Ho and Walker [39] and were purified by repeated crystallizations from absolute ethanol. DMT-*N*-oxide (DMT-NO, X)·H_2O was prepared and purified by the method of Fish *et al.* [36]. Heptafluorobutyrylimidazole (HFBI) was a gift from the Pierce Chemical Co. Rockford, IL. [5-^{3}H]DMT was prepared by the New England Nuclear Corp., Boston, MA, and had a specific activity of 155 mCi/mg. [5-^{3}H]DMT-NO (sp. act. = 155 mCi/mg) was prepared from [5-^{3}H]DMT by the method of Fish *et al.* [36]. All other reagents were obtained from commercial sources and were of the highest available purity.

Preparation of rat whole brain homogenate. For each study, three (3) adult male Sprague–Dawley rats (Southern Animal Farms, Prattville, AL) weighing between 250 and 300 g each were decapitated and the brains were rapidly excised. The pooled brains were homogenized in 40 ml of ice-cold 0.32 M sucrose in 0.20 M phophate buffer, pH 7.4, using a glass homogenizer and a Teflon pestle (clearance of 0.25 mm). The crude homogenate was diluted to 100 ml with phosphate buffer, and an aliquot was saved for determination of protein. The homogenate was used in the incubation assays.

A second group of three rats was treated with the MAOI iproniazid phosphate (100 mg/kg in isotonic saline, pH 7.4, by intraperitoneal injection) 3 hr prior to death and work-up as described previously.

Metabolism of DMT in rat whole brain homogenates. Incubation mixtures contained 1.0 ml of [5-^{3}H]DMT (6.0×10^{-8} M final concentration, giving 4.0×10^9 d.p.m./ml) or 1.0 ml of [5-^{3}H]DMT-NO (2.7×10^{-8} M final concentration, giving 1.7×10^9 d.p.m./ml) in phosphate buffer (pH 7.4). Sufficient 'cold' substrate was added from a 1.0×10^{-3} M stock solution of DMT when a higher concentration (2.0×10^{-5} M) was studied. Phosphate buffer (pH 7.4) was added, to a volume of 2.0 ml, and reactions were initiated by the addition of 1.0 ml of whole brain homogenate which contained, on the average, 7.2 mg protein/ml, as determined by the method of Lowry *et al.* [40]. Total incubation volumes were 3.0 ml. Anaerobic metabolism was simulated by gently passing high purity N_2 through the reaction mixtures at 4° for 10 min and then sealing the tubes with rubber closures. Boiled enzyme blanks were used as controls in all studies.

Incubations were conducted at 37° with gentle shaking. To determine the levels of the metabolites with time, samples were incubated for 30-, 60- and

120-min intervals. The samples were removed and immediately frozen at −76°, lyophilized (Virtis Unitrap), and the residue extracted with three 1.0-ml aliquots of boiling absolute ethanol. The combined ethanol extracts were concentrated to 1.0 ml by a stream of high purity dry N_2 and cooled to 0°. The samples were centrifuged (Universal Clinical Centrifuge) and 100-μl aliquots of the supernatant fractions were spotted on thin-layer chromatography (t.l.c.) plates (Quantum LQD, silica gel.) Carrier DMT, NMT, 2-MTHBC, DMT-NO and IAA were concurrently spotted with the samples, and the plates were developed by ascending chromatography in each of the following equilibrated solvent systems: (A) methanol–NH_4OH (1.0 M), 5:1, and (B) isopropanol–ethyl acetate–NH_4OH (conc.), 80:20:4. The plates were air dried, and 2-MTHBC was visualized with ultraviolet light. Indolic constituents were visualized by spraying the plates with Erhlich's reagent [41]. Areas corresponding to the appropriate compounds were scraped into counting vials and eluted with 1.0 ml of absolute ethanol. The remainder of the plate was divided into 1.0-mm sections which were similarly scraped and eluted. Scintillation mixture (10.0 ml) was added, and the samples were vigorously agitated and counted (Packard model 3375 liquid scintillation spectrometer). The c.p.m. values were converted to d.p.m. and then to μmoles of product. The overall extraction efficiency of added radioactivity was >90 per cent.

Metabolism of 2.0×10^{-5} M *DDMT.* In order to confirm product identification using g.c./m.s., incubations of 2.0×10^{-5} M DDMT were also conducted in whole rat brain homogenates obtained from animals which had not been pretreated with iproniazid phosphate. The incubations were conducted as described above, except samples were withdrawn only after the 120-min time interval. To determine the contribution of HCHO to the formation of the THBC's, identical incubations were conducted in the presence of 1.0×10^{-4} M dimedone, which served as a HCHO trap. The samples and boiled enzyme blanks following incubation were placed in ice and 1.0 ml of 70% $HClO_4$ was added. These were centrifuged (Universal Clinical), to remove precipitated protein, and the supernatant fractions were decanted. The pH of the samples was adjusted to 12 in an ice bath by the addition of 45% KOH and centrifuged to remove precipitated $KClO_4$. The basic aqueous phase containing DMT and its metabolites was then extracted with two 6.0-ml aliquots of CH_2Cl_2. The pooled organic layer was dried with 2.0 g of Na_2SO_4 and evaporated in three portions in a 15 ml conical flask. The resulting residue was derivatized with HFBI to prepare the corresponding heptafluorbutyryl (HFB) derivatives of the products [42]. This method did not extract IAA or DMT-NO and, thus, the formation of these compounds could not be confirmed by this technique. The extraction efficiency for DMT, NMT, TA, THBC and 2-MTHBC is 90 per cent using this method [43–45]. In order to further identify DMT-NO, two additional solvent systems were utilized [chloroform–methanol–NH_4OH (conc.), 60:10:1 and *n*-butanol–acetic acid (glacial)–water, 8:1:1]. The *N*-oxide was also identified by reacting the eluted sample from the thin-layer chromatography plates, corresponding to the R_f for DMT-NO, with zinc and acetic acid [36]. The reduced *N*-oxide was rechromatographed in the previously described solvent systems and identified as DMT.

Gas chromatographic/mass spectrometric (g.c./m.s.) identification of the metabolites of DDMT from whole rat brain homogenate extracts. Authentic reference standards of DMT, DDMT, NMT, TA, DTA, 2-MTHBC, THBC and TDBC were quantitatively converted to their corresponding HFB derivatives [42–45] for g.c./m.s. analysis. The g.c./m.s. chracteristics of these compounds were determined using a Hewlett Packard 5985 GC/MS equipped with a data analysis system. The g.c. was conducted on a Supleco 4′, 2 mm internal diameter, glass column containing 2% SP-2250 on 100–200 mesh Chromasorb-W-HP. A stepped temperature program was used to obtain efficient separation; 150° initial T rise increased to 30°/min to 250°. High purity helium was used as the carrier gas and a flow rate of 40 ml/min was maintained throughout the run. Electron impact (EI) mass spectra of the compounds studied were recorded by total ion (TI) monitoring of the effluent and were characterized with respect to their base peaks (normalized to 100 per cent) and other prominent secondary mass fragments.

The retention times were recorded for each compound and the chosen mass fragments were monitored in the selected ion monitoring (SIM) mode to determine the ratios of the selected ions. The mass spectrometer was tuned daily, using the *m/e* peaks 69.0, 219.0 and 502.0 atomic mass units (amu) of the calibration standard perfluorotributylamine for the EI-TI studies. For the SIM studies, the instrument was detuned for the 69.0 peak to maximize the sensitivity at the lower mass ranges. Reference standards were examined in the SIM mode, recording retention times and ion ratios, prior to and following injection of the derivatized rat brain extract. The 2.0×10^{-5} M solution of DDMT used in the incubation was also assayed by this method to assure purity of the sample.

RESULTS

The results of the metabolism assays for [5-^{3}H]DMT and [5-^{3}H]DMT-NO following a 30-min incubation period are presented in Table 1. The R_f values for the solvent systems used in this study are presented in Table 2.

Incubation of 6.0×10^{-8} M [5-^{3}H]DMT for 30 min with rat whole brain homogenate yielded IAA, NMT, 2-MTHBC and DMT-NO as metabolites. The major metabolite was IAA. Incubation of DMT at a higher concentration (2.0×10^{-5} M) also gave IAA, NMT, 2-MTHBC and DMT-NO as metabolites. However, at this concentration DMT-NO was the major metabolite at 30 min.

When 6.0×10^{-8} M [5-^{3}H]DMT was incubated with whole brain homogenate obtained from rats pretreated with the MAO inhibitor iproniazid, IAA formation was inhibited by 83 per cent. However, the formation of both NMT and DMT-NO was inhibited by 90 per cent and no 2-MTHBC was detected.

Table 1. Metabolites formed in 30-min incubations of [5-^{3}H]DMT and DMT-NO in whole rat brain homogenates

Substrate	Concn (M)	Iproniazid pretreatment	Aerobic or Anaerobic	Metabolites (μmoles/min/mg protein $\times 10^8$) 2-MTHBC	NMT	DMT	DMT-NO	IAA
[5-^{3}H]DMT	6.0×10^{-8}	No	Aerobic	0.79	1.22		1.85	20.5
	6.0×10^{-8}	Yes	Aerobic	0.00	0.12		0.19	3.47
	2.0×10^{-5}	No	Aerobic	0.06	10.90		33.00	1.95
[5-^{3}H]DMT-NO	2.7×10^{-8}	No	Aerobic	0.09	0.92	0.42		2.74
	2.7×10^{-8}	No	Anaerobic	1.00	2.50	2.52		2.01

Table 2. Solvent systems for thin-layer chromatographic analyses of DMT and DMT-NO metabolites

Solvent systems	Compound and R_f DMT-NO	DMT	NMT	IAA	2-MTHBC
Methanol, NH_4OH (1 M), 5:1	0.74	0.52	0.32	0.92	0.26
Isopropanol, ethylacetate, NH_4OH (conc.), 80:20:4	0.09	0.37	0.19	0.14	0.17
Chloroform, methanol, NH_4OH (conc.), 60:10:1	0.42	0.73	0.52		
n-Butanol, acetic acid (glacial), water, 8:1:1	0.61	0.50	0.70		

Incubation of 2.7×10^{-8} [5-^{3}H]DMT-NO yielded IAA, DMT and 2-MTHBC as metabolites. Anaerobic incubation with [5-^{3}H]DMT-NO as substrate stimulated DMT and NMT production, while IAA formation remained essentially unchanged. The formation of 2-MTHBC was also stimulated under these conditions.

The metabolism of [5-^{3}H]DMT at a concentration of 6.0×10^{-8} M as a function of time is presented in Fig. 2. The formation of all metabolites had essentially peaked at or before 30 min with levels of NMT, DMT-NO and 2-MTHBC having decreased at the end of the 2-hr incubation period. Production of IAA continued to increase with time.

Incubation of 2.0×10^{-5} M [5-^{3}H]DMT with time (Fig. 3) showed that *N*-oxide formation was maximal at or before 30 min and was the major metabolite when measured at this time and concentration of DMT. Production of NMT peaked at 1 hr and then declined sharply. IAA was the major metabolite at the end of this 2-hr incubation period. However, 2-MTHBC formation was observed to be still increasing.

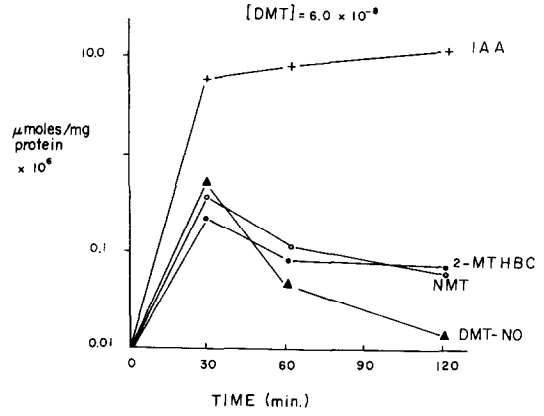

Fig. 2. The metabolism of 6.0×10^{-8} M [5-^{3}H]DMT in whole rat brain homogenate as a function of time.

For the g.c./m.s. analyses of DDMT metabolism, electron impact (EI) mass spectra of the compounds studied were recorded as described. The retention times were noted and the chosen mass fragments were monitored in the selected ion monitoring (SIM) mode for the corresponding deuterated metabolites. Figure 4 illustrates the retention times and SIM mode results for the analyses. The total ion mass spectra for TA, DTA, TDBC [44, 45], THBC [44–46], NMT [13, 47], DMT, DDMT [48] and 2-MTHBC [49] have been reported previously. The ions 328.2 and 342.1 were used to monitor the presence of deuterated TA and NMT, while the ion fragments at 145.0 and 115.0 were used to monitor for deuterated 2-MTHBC. The ions 145.0, 372.0 were monitored for the presence of TDBC. The ions 60.1 and 131.1 were monitored for DMT. Table 3 illustrates the analysis of DDMT and its metabolites, showing the correlation of the peaks from the extract with the standards. Peaks were also compared for ion ratios as a further means of positive identification.

The g.c./m.s. analyses confirmed the findings of the experiments using [5-^{3}H]DMT with the identification of deuterated NMT and 2-MTHBC as metabolites of DDMT (Fig. 5). However, trace amounts of deuterated TA and THBC were also identified as metabolites by the g.c./m.s. method, having gone undetected in the [5-^{3}H]DMT assays. IAA and DMT-NO were identified by t.l.c. (Table 2).

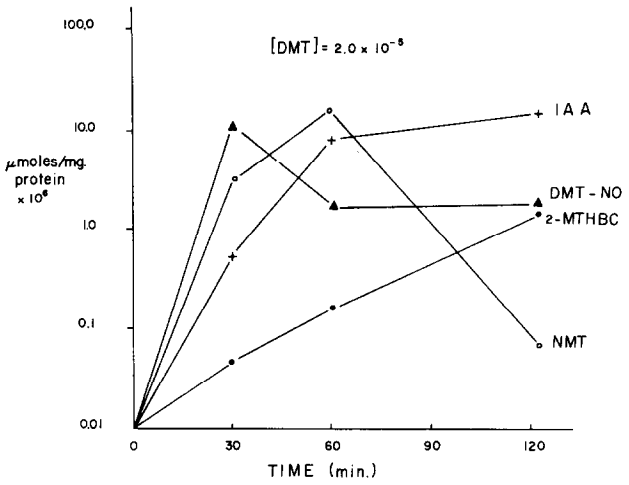

Fig. 3. Metabolism of 2.0×10^{-5} M [5-^{3}H]DMT in whole rat brain homogenate as a function of time.

Gas chromatographic/mass spectrometric analysis of incubation mixtures which contained dimedone (Fig. 5) showed that approximately 50 per cent of the β-carbolines identified in the assay could be accounted for by the condensation of HCHO with NMT and TA via a Pictet–Spengler condensation [50].

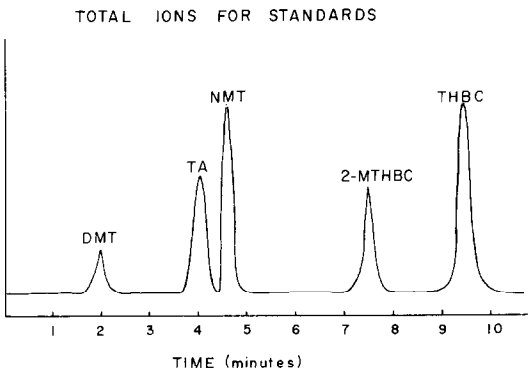

Fig. 4. Total ion scan for standards used in g.c./m.s. analyses.

DISCUSSION

The formation of an *N*-oxide and a secondary amine from DMT coincides with the known metabolism of many tertiary amines [51]. For example, Bickel [52] and Willi and Bickel [53] have demonstrated that four simultaneous reactions occur in liver microsomes during the metabolism of tertiary amines, i.e. *N*-oxidation, *N*-oxide reduction, *N*-oxide demethylation and tertiary amine demethylation. The findings of our present study of DMT metabolism are in agreement with such a metabolic process occuring in rat brain. In the first *in vitro* study of DMT metabolism [36], using mouse liver homogenate as the enzyme source, DMT-NO was identified as the major metabolite. A mitochondrial fraction from liver converted DMT to DMT-NO and IAA but did not metabolize DMT-NO when this compound was used as a substrate. It was concluded that the formation of the *N*-oxide was enzymatic and was the major metabolite formed in a crude microsomal preparation from mouse liver. It was further concluded that the *N*-oxide was not an intermediate in the oxidative deamination of DMT by mouse liver mitochondria. However, it was observed that the *N*-

Table 3. Gas chromatographic/mass spectrometric analyses of standards and DDMT and its metabolites

				DDMT metabolism		
	Retention time standards*	Ions monitored	Ion ratios (%)	Retention time of peak observed*	Ions monitored	Ion ratios (%)
DDMT	2.0	60.1, 131.1	100:8 ± 2	2.0	60.1, 131.1	100:8
DTA	4.1	342.1, 328.2	100:91 ± 6	4.1	342.1, 328.2	100:91
NMT	4.6	339.0, 326.2	100:28 ± 5	4.6	342.1†, 328.2	100:28
2-MTHBC	7.5	143.1, 115.0	100:15 ± 5	7.5	145.1‡, 115.0	100:15
TDBC	9.5	145.0, 115.0, 372.0	100:33 ± 2:4 ± 4	9.5	145.0, 115.0, 372.0	100:33:4

* Compounds were analyzed as heptafluorobutyryl derivatives.
† Ions for tetradeutero-NMT.
‡ Ions for tetradeutero-2-MTHBC.

oxide is metabolized to give traces of other indoles in whole liver homogenate, but these products were not identified. Subsequently, Barker *et al.* [38] demonstrated that DMT-*N*-oxide is metabolized in rat liver microsome preparations obtained from animals pretreated with iproniazid to yield DMT, NMT and HCHO, demonstrating the interconversion of DMT and its *N*-oxide. Szara and Axelrod [24] reported that DMT is metabolized in rabbit liver microsomes obtained from animals pretreated with iproniazid to give TA, NMT, DMT-NO, 6-hydroxy-DMT and 6-hydroxy-DMT-NO. The formation of NMT was accompanied by the liberation of HCHO. While 6-hydroxylation is a recognized pathway of indole metabolism in the periphery, it apparently does not occur in brain [38, 54].

Metabolism of DMT by MAO. With respect to the formation of IAA in assays of DMT metabolism, both *in vivo* and *in vitro*, it is probable that a large portion of the IAA arises via the oxidative deamination of NMT rather than by direct action of MAO on DMT. The relative rate of NMT oxidation by MAO has been measured as being 9 times greater than that for DMT and 280 times greater than that for DMT-NO, the *N*-oxide being essentially resistant to metabolism by this enzyme under aerobic conditions [32, 36]. DMT *per se* is not only a poor substrate for MAO [31, 33, 34] but is itself an MAOI [33, 34, 55]. In the present study, the formation of IAA was inhibited by 83 per cent when 6.0×10^{-8} M [5-^{3}H]DMT was incubated with whole brain homogenates obtained from rodents pretreated with the MAOI iproniazid. However, NMT and DMT-NO formation was inhibited by 90 per cent and no 2-MTHBC formation occured. Accordingly, the reported extension of DMT half-life in brain [27, 28] and potentiation of its behavioral effects [27–29] following iproniazid pretreatment may be due to inhibition of the enzymes responsible for demethylation as well as *N*-oxidation of DMT, rather than strictly MAO inhibition.

Formation of THBCs. Hahn and Ludewig [56] first demonstrated the spontaneous condensation of tryptamines and aldehydes to form tetrahydro-β-carbolines under physiological conditions. The formation of 2-MTHBC and THBC *in vitro* has been demonstrated repeatedly in incubations of the methyl donors 5-methyl-tetrahydrofolate (5-MTHF) and SAM with NMT and TA, respectively [5]. The formation of the β-carbolines occurs via the enzymatic formation of HCHO from either methyl donor. The HCHO thus formed condenses non-enzymatically with the indole substrates NMT and TA via a Pictet–Spengler reaction [50]. This mechanism is supported by the fact that semicarbazide trapping of HCHO produced from the methyl donors eliminates the formation of THBCs *in vitro* [57]. The present study has demonstrated the *in vitro* formation of 2-MTHBC and trace amounts of THBC during the metabolism of DMT. These compounds may have resulted from the condensation of the other DMT metabolites, NMT, trace amounts of TA, and HCHO. Free HCHO is produced during incubations of DMT with rodent brain [38] and liver fractions [24, 38]. In the present study, addition of dimedone to the DDMT metabolism assays did decrease, although not eliminate, the formation of 2-MTHBC and THBC, indicating that a portion of the THBCs was formed via non-enzymatic condensation of NMT and TA with HCHO. However, the possibility that THBC may also be formed via the demethylation of 2-MTHBC must also be considered.

It is of interest to note that, mechanistically, intermediates in the formation of THBCs from an amine and HCHO and those proposed in the demethylation of tertiary amines, by either direct C-hydroxylation or *N*-oxide rearrangement, are identical (Fig. 6). Thus, the elimination of HCHO is not necessarily a prerequisite in the formation of THBCs from DMT. Both mechanisms lead to the formation of an iminium ion which can cyclize to form tetrahydro-β-carbolines.

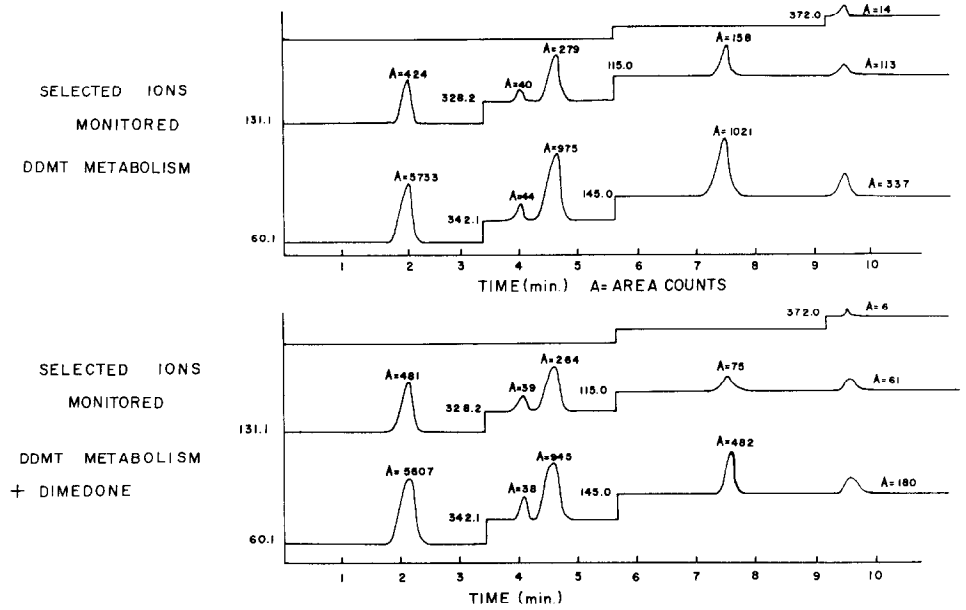

Fig. 5. SIM scans of DDMT and DDMT plus dimedone metabolism assays.

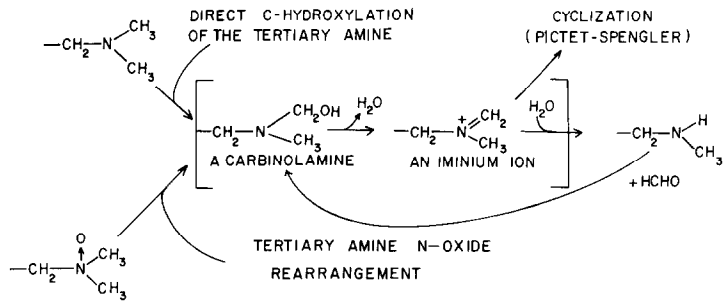

Fig. 6. Mechanisms for the demethylation of tertiary amines and tertiary amine *N*-oxides illustrating the intermediates which are identical with those proposed in the Pictet–Spengler reaction.

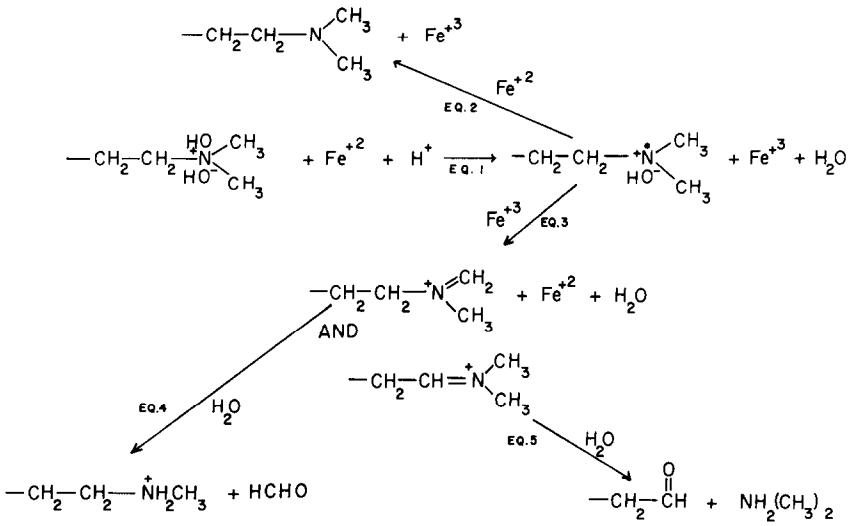

Fig. 7. Mechanisms for the biomimetic reactions of tertiary amine *N*-oxides under reductive conditions.

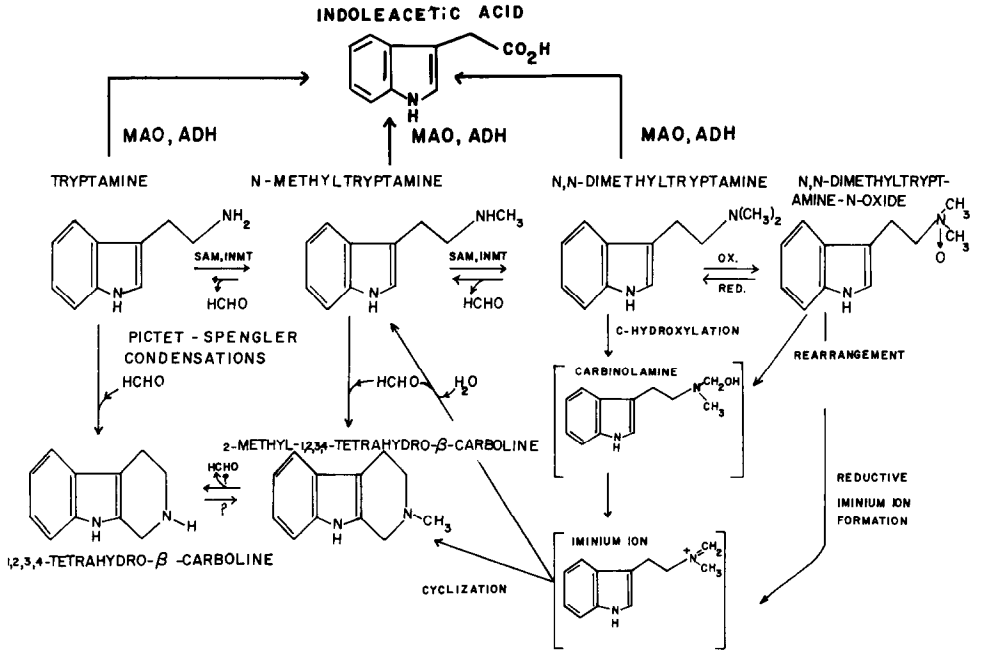

Fig. 8. Proposed pathway for DMT synthesis and metabolism in brain tissue. ADH = aldehyde dehydrogenase.

Metabolism of DMT-NO. The metabolism of DMT-NO leads to the formation of 2-MTHBC, NMT, IAA and DMT. Since the *N*-oxide appears to be the major intermediary metabolite in *in vitro* incubations [31, 38], it may play a pivotal role in the overall metabolism of DMT *in vivo*. As mentioned previously, four reactions are known to occur simultaneously during the metabolism of tertiary amines, i.e. *N*-oxidation, *N*-oxide reduction, tertiary amine demethylation, and *N*-oxide demethylation. At present. the relative contributions of these four reactions to the metabolism of DMT and DMT-NO are not known with any certainty and must await further research efforts to answer this question.

The increased formation of 2-MTHBC, NMT and DMT from DMT-NO under anaerobic conditions (Table 1) may proceed by mechanisms analogous to the biomimetric reactions of *N*-oxides with reduced iron (Fe^{2+}) [58–64]. Figure 7 illustrates this mechanism. Ferris *et al.* [59] showed that the demethylation of *N*-oxides proceeded via the aminium radical ion (equation 1) which, in turn, yields a tertiary amine (equation 2) and the iminium ion (equation 3). The iminium ion may then be hydrolyzed (equation 4) to a secondary amine and HCHO. This mechanism is identical with those proposed by other investigators for the reaction of *N*-oxides with Fe^{2+} [58, 61, 62] under biomimetic conditions.

Using anaerobic conditions, a large proporiton of enzymes would be in their reduced state *in vitro*. It is thus possible that the reduced prosthetic groups of microsomal enzyme systems catalyze the formation of the aminium radical ion (equation 1, Fig. 7) and thus the reduced tertiary amine (equation 2) and the iminium ion (equation 3) of DMT. *N*-oxide reduction, for example, is known to occur *in vitro* via the reduced form of cytochrome P-450 [65–67]. If an iminium ion is formed with the α-carbon of DMT, it could be hydrolyzed to yield indole-3-acetaldehyde and dimethylamine, analogous to equation 5 (Fig. 7) Ghosal and Mukherjee [68] noted such a reaction in the Fe^{2+} catalyzed rearrangement of 5-methoxy-DMT-*N*-oxide, where 5 methoxy-DMT, 5-methyoxy-NMT, HCHO, 6-methoxy-2-MTHBC and 5-methoxy-indole-3-acetaldehyde were isolated as products. Under the appropriate conditions, if DMT-NO undergoes such a reaction *in vitro*, it may account for a portion of the IAA formed from DMT. However, the major portion of the IAA most likely arises via the oxidative deamination of NMT formed via the demethylation of DMT and DMT-*N*-oxide.

Furthermore, an iminium ion formed from DMT-NO, as in equation 3, would provide an intermediate identical with that proposed in the Pictet–Spengler reaction [50] (Fig. 6). This intermediate is also proposed in the Fe^{2+} catalyzed rearrangement of other indoleamine-*N*-oxides to tetrahydro-β-carboline compounds [58, 68]. The iminium ion thus formed from DMT-NO could then cyclize to form 2-MTHBC. The results of the incubation of DMT-NO under anaerobic conditions are in agreement with the above hypotheses and the mechanisms shown in Fig. 7.

In conclusion, the present study has demonstrated that the metabolism of DMT in whole rat brain homogenate leads to the formation of DMT-NO, NMT, IAA and 2-MTHBC. Trace amounts of TA and THBC are also apparently formed. The major end metabolite of DMT is IAA. However, DMT-NO and NMT are most likely major intermediates. Our results also indicate that administration of the MAOI iproniazid to animals prior to the addition of DMT to brain homogenates inhibits not only MAO but also the enzymes responsible for the formation of key intermediates in the metabolism of DMT, i.e. DMT-NO and NMT.

Mechanisms for the formation of 2-MTHBC and THBC *in vitro* have been offered. Although the inclusion of dimedone in the reaction medium inhibited the formation of the β-carbolines from DMT, it did not completely eliminate their production, suggesting that the formation of these compounds may occur by mechanisms other than those requiring the dissociation of HCHO *in vitro*.

Drawing on the results of the present study and those of others in both the biosynthesis and metabolism of DMT, a pathway is proposed (Fig. 8). Since DMT may be involved in both normal [43] and/or abnormal [3, 4] brain function, the above pathway may be useful in studying these two possibilities. The idea of a 'cyclic' metabolic pathway in the mammalian CNS raises new questions with respect to the possible involvement of these compounds in brain function.

Since it has been suggested that DMT as well as the β-carbolines [43, 45] may act as neuroregulatory agents in the CNS, it is imperative that we understand the metabolism of these compounds as completely as possible. The identification of THBC as an *in vivo* constituent of rat brain has been reported recently [44–46, 69]. THBC and trace amounts of 2-MTHBC have also been identified recently in rat adrenal glands [69].

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