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STUDY OF METABOLISM OF PSYCHOTOMIMETIC INDOLEALKYLAMINES BY RAT TISSUE EXTRACTS USING LIQUID CHROMATOGRAPHY

BALVANT R. SITARAM,* RERNGJIT TALOMSIN, GRAEME L. BLACKMAN and WILLIAM R. MCLEOD

School of Pharmaceutical Chemistry, Victorian College of Pharmacy Ltd., Parkville, Victoria, Australia 3052

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Abstract—The use of a series of liquid chromatographic techniques involving cation-exchange, reverse-phase and normal-phase chromatography has permitted the separation and characterisation of a number of metabolites of the psychotomimetic indolealkylamines *N*,*N*-dimethyltryptamine and 5-methoxy-*N*,*N*-dimethyltryptamine which were isolated following incubation of these compounds with rat tissue extracts. In liver, kidney and brain tissue extracts the routes of metabolism identified included oxidative deamination, N-demethylation, O-demethylation and N-oxidation. The quantitative significance of individual routes of metabolism in these tissues was assessed using *N*,*N*-dimethyltryptamine as a substrate.

A number of psychotomimetic indolealkylamines have now been identified in mammalian tissues and body fluids including those of humans [1–7]. Despite evidence that *N*,*N*-dimethyltryptamine (DMT), 5-hydroxy-*N*,*N*-dimethyltryptamine (5OHDMT), and 5-methoxy-*N*,*N*-dimethyltryptamine (5MeODMT) are very rapidly metabolized *in vivo* [8–11], the routes of metabolism involved remain incompletely understood. While potential pathways for the metabolism of those indolealkylamines have been suggested in earlier reports, many studies remained only qualitative, with the identification of purported metabolites based on the comparison of chromatographic behaviour with standards following either paper or thin-layer chromatography [12, 13].

In recent studies Barker *et al.* [14] have examined the *in vitro* metabolism of DMT using gas chromatographic–mass spectroscopic and radioisotopic–thin layer chromatographic techniques for the quantitation and identification of individual metabolites. However, these studies were restricted to the metabolism of DMT by rat brain homogenates. We now report the use of liquid chromatographic–fluorometric techniques for the separation, identification, and quantitation of metabolites of DMT and 5MeODMT formed in the presence of a number of rat tissue extracts, namely those of liver, kidney and brain. A number of these metabolites were identified by a comparison with authentic standards of their chromatographic behaviour on cation-exchange, reverse-phase and normal-phase columns. Where possible, additional confirmation of identity has been obtained by stop-flow fluorescence spectroscopic analysis. The relative significance of individual routes of metabolism of DMT in various tissues was examined.

* All correspondence should be sent to: Balvant R. Sitaram, Ph.D., School of Pharmaceutical Chemistry, Victorian College of Pharmacy, 381 Royal Parade, Parkville, Victoria, Australia 3052.

MATERIALS AND METHODS

Materials

N-Methyltryptamine (NMT), *N*,*N*-dimethyltryptamine (DMT), 5-methoxy-*N*,*N*-dimethyltryptamine (5MeODMT), indoleacetic acid (IAA), 5-methoxyindoleacetic acid (MeOIAA) and D-glucose-6-phosphate were purchased from the Sigma Chemical Co.; tryptamine hydrochloride was from the Aldrich Chemical Co.; nicotinamide adenine dinucleotide phosphate (NADP) and glucose-6-phosphate dehydrogenase (yeast) Grade 1 were from the Boehringer Chemical Co.; and indoleacetaldehyde (IAC) was from the Regis Chemical Co. 1,2,3,4-Tetrahydro-β-carboline (THBC), 2-methyl-1,2,3,4-tetrahydro-β-carboline (MTHBC), 5-methoxy-*N*-methyltryptamine (5MeONMT) and *N*,*N*-dimethyltryptamine-*N*-oxide (DMT-NO) were donated by Dr. S. A. Barker, University of Alabama. All solvents used for chromatography were of analytical purity and glass distilled prior to use.

Instrumentation

Chromatography of the indoleamines and their metabolites was performed on a Perkin–Elmer Series 3B liquid chromatograph. Separations were achieved using a strong cation exchange column (Whatman Partisil 10 SCX 25 cm × 4.6 mm i.d.) using a mobile phase consisting of methanol–0.08 M acetic acid/ammonia buffer, pH 4.4 (30:70) with a flow rate of 1.5 ml/min. Additional separations were achieved using normal chromatography on a silica column under conditions previously described [15].

Indoleacetic acid, indoleacetaldehyde and 5-

methoxyindoleacetic acid were separated by reverse-phase chromatography on an ODS column (DuPont Zorbax, ODS 25 cm × 4.6 mm i.d.) with a mobile phase consisting of methanol–0.08 M acetic acid/ammonia buffer, pH 4.5 (50:50) and with a flow rate of 1 ml/min. The spectroscopic detectors that were used included a Perkin–Elmer 650-10S fluorescence spectrometer and a Perkin–Elmer 3000 fluorescence spectrometer.

Spectroscopic studies

Fluorescence spectroscopy was conducted on the various metabolites by halting the flow of the mobile phase as each component entered the detector flow cell. Excitation and emission spectra were recorded and compared with similar spectra obtained from authentic standards.

Preparation of tissue fractions

Homogenate fraction. Adult male Sprague–Dawley rats (150–250 g), fed *ad lib.* and maintained in a 12 hr:12 hr light:dark cycle, were decapitated (10:00–11:00 a.m.) and the tissues were rapidly excised and chilled on ice. All subsequent steps were carried out at 0–4°.

All tissues (except brain) were homogenised in 10 vol. of 0.25 M sucrose in 0.01 M potassium phosphate buffer, pH 7.4, using a Potter–Elvehjem homogeniser with 0.25 mm clearance. Brain tissue was homogenised in 10 vol. of 0.32 M sucrose in 0.01 M potassium phosphate buffer, pH 7.4.

Post-mitochondrial fractions. The crude tissue homogenate was centrifuged at 10,000 *g* for 20 min. The supernatant fraction obtained served as the post-mitochondrial fraction.

Microsomal fractions. The microsomal fraction was prepared by centrifugation of the post-mitochondrial supernatant fraction at 100,000 *g* for 60 min. The supernatant fraction was removed, and the pellet was washed with 0.25 M sucrose in 0.01 M potassium phosphate buffer and resuspended in buffer to give a final protein concentration of about 10 mg/ml. Protein was estimated by the method of Lowry *et al.* [16].

Assay for formation of metabolites

All reactions were carried out in 10-ml conical flasks. The reaction mixture ordinarily contained 40 μmol potassium phosphate buffer, pH 7.4; 1 μmol substrate; 0.27 μmol NADP; 4 μmol glucose-6-phosphate; 0.67 μmol magnesium chloride; 4 μl glucose-6-phosphate dehydrogenase; and 200 μl of tissue fraction in a final volume of 600 μl. All additions were made at 0° in an ice bath. After incubation at 37° for 30 min (Dubnoff metabolic shaking incubator), a 300-μl aliquot of the reaction mixture was transferred to a glass tube and cooled to 0° in an ice bath. The reaction was terminated by the addition of 700 μl of absolute ethanol and, following vortexing and centrifugation at 3000 *g* for 15 min, aliquots of the supernatant fraction were analysed for the presence of metabolites.

Determination of recoveries

Incubation mixtures containing rat liver homogenates and 2 nmol of IAA, IAC, DMT-NO or NMT were analysed immediately following extraction with ethanol. The recoveries of these potential metabolites were determined by a comparison of the relative fluorescence obtained following chromatography on the SCX column with appropriate standards. The percent recoveries for IAA, IAC, DMT-NO, and NMT were 98.5 ± 0.6, 92.5 ± 0.8, 98.6 ± 1.4 and 90.0 ± 0.6 respectively (mean ± SEM for three determinations).

Separation and identification of metabolites

Samples of the supernatant fraction (10 μl) were chromatographed on the SCX column, and the metabolites were detected by fluorescence spectroscopy as described above. Metabolites were initially characterised by the comparison of retention time with authentic standards. Additional characterisation was achieved by stop-flow spectroscopic analysis, both the excitation and emission spectra being compared to those of authentic standards.

To further confirm the identity of the metabolites, those eluent fractions from the SCX column containing individual metabolites were collected in conical centrifuge tubes and lyophilised using a high vacuum unit (Dynavac, Australia model FD2). Samples in the freeze dryer were shielded from light. The residue was reconstituted in 500 μl of methanol, and 100-μl aliquots were then subjected to secondary chromatography on either the Zorbax ODS column or the silica column as described above. The chromatographic characteristics of the metabolites were then compared with those of authentic standards.

Quantitation of metabolites of DMT

Quantitation of the metabolites of DMT-NO and NMT was based on a comparison of relative fluorescence (peak height) with those of authentic standards following separation on the SCX column. To achieve adequate resolution of the metabolites IAA and IAC prior to quantitation, aliquots (100 μl) of the SCX column eluent fractions containing these metabolites were subjected to secondary chromatography on the ODS column. Analysis of extracts prepared from no-substrate, no-incubation and no-homogenate controls indicated an absence of any coeluting interferences which could affect the quantitation of these metabolites.

RESULTS

We have described liquid chromatographic techniques for the separation and on-line fluorescence detection of the psychotomimetic indolealkylamines DMT, 5MeODMT and 5OHDMT and a number of derivatives including their analogous tetrahydro-β-carbolines [15].

The techniques used in this study for the separation, detection and characterisation of the metabolites of DMT are based on these earlier developments. Additional separations of the indole acid and indoleacetaldehyde derivatives of DMT and 5MeODMT were achieved on an ODS column as described in Materials and Methods. To facilitate the recovery of the individual metabolites from the column eluent fractions, the SCX and ODS column mobile phases were modified to incorporate the use

of ammonium acetate based buffers. The sublimation of ammonium acetate on lyophilisation resulted in residues free of salts.

The conditions of assay including the concentration of substrates required for the optimum synthesis of metabolites were pre-determined using liver tissue homogenates as a source of enzyme activity. Under the conditions described, the production of metabolites of DMT and 5MeODMT increased linearly with time of incubation and homogenate protein concentration within the limits 0–15 mg/ml.

Identification of metabolites of DMT

A number of metabolites, designated Metabolites A–E (Fig. 1a), were formed from DMT following its incubation in the presence of rat liver homogenate. Metabolites A and B were detected when DMT was incubated with rat liver homogenates but were formed in greatly reduced quantities when DMT was incubated with PMS fractions from rat liver. The synthesis of both these metabolites was independent of the availability of exogenous NADP(H) and was inhibited markedly in animals pretreated with the monoamine oxidase inhibitor iproniazid (Fig. 1b). On the basis of these observations and their chromatographic characteristics on the SCX column, the two metabolites were identified as IAC and IAA. Further confirmation of identity was obtained following their isolation from the SCX column effluent. Aliquots of those eluent fractions containing Metabolites A and B were subjected to secondary chromatography on an ODS column as described in Materials and Methods. The retention time characteristics of the two metabolites were identical to those of authentic IAA and IAC (Fig. 2). The excitation and emission spectra recorded following complete separation of the metabolites on the ODS column were also identical to those of the appropriate authentic standards (λ_{ex} 275 nm, λ_{em} 353 nm for IAA and λ_{ex} 257 nm, λ_{em} 350 nm for IAC).

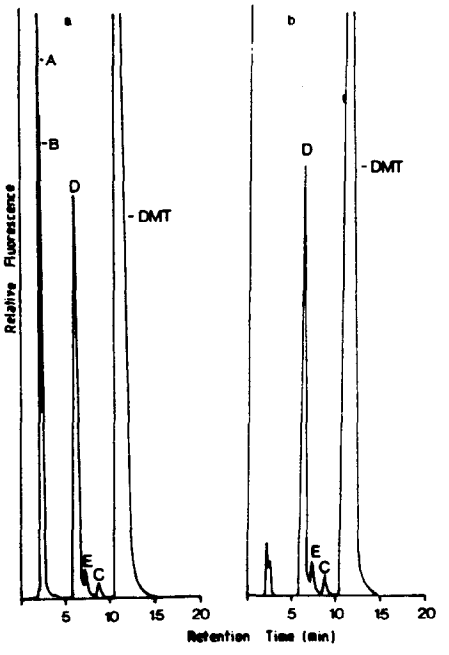

Fig. 1. Chromatograms of DMT and its metabolites formed (a) in the presence of rat liver homogenate and (b) in the presence of liver homogenate from rats pretreated with iproniazid (100 mg/kg i.p. 2 hr prior to sacrifice). DMT was incubated in the presence of rat liver homogenate as described in Materials and Methods. Ten microliters of the final extracts were applied to the SCX column. Compounds were detected by fluorescence spectroscopy (λ_{ex} 280 nm, λ_{em} 357 nm).

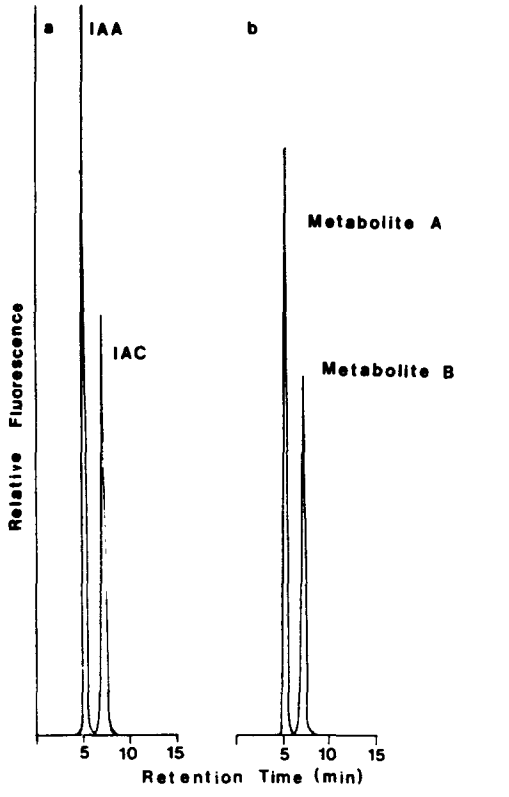

Fig. 2. Comparison of chromatography of Metabolites A and B formed in the presence of liver extracts with that of IAA and IAC. A sample (100 μl) of the SCX column eluent fractions containing Metabolites A and B was applied to the ODS column. Authentic samples of IAA and IAC (50 pmol in 10 μl of methanol) were similarly chromatographed. Compounds were detected by fluorescence spectroscopy (λ_{ex} 275 nm, λ_{em} 350 nm).

In contrast to Metabolites A and B, the synthesis of Metabolite D showed a marked dependence on the availability of exogenous NADP(H); [in the absence of NADP(H) the rate of synthesis of DMT-NO was reduced by 93%]. In addition, the enzymes responsible for its synthesis were present in the homogenate, in the PMS fraction, and in the 100,000 *g* microsomal fraction of rat liver. On the basis of these observations and its chromatographic characteristics on the SCX column, Metabolite D was identified as DMT-NO. Further confirmation of its identity was obtained by stop-flow spectroscopic analysis following chromatography on the SCX column. Both the excitation and emission spectra of Metabolite D were identical with those of authentic DMT-NO (Fig. 3). In addition, following its isolation from the SCX column eluent and secondary chromatography on the silica column, the metabolite again

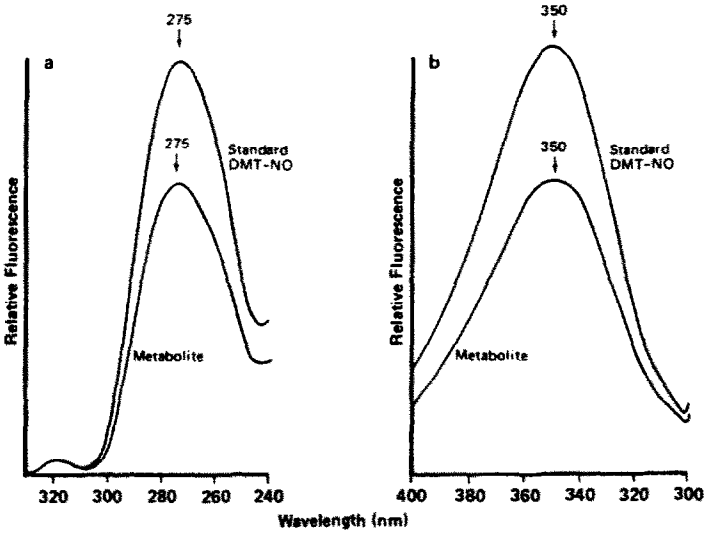

Fig. 3. Fluorescence spectra of authentic DMT-NO and Metabolite D. DMT was incubated in the presence of rat liver homogenate as described in Materials and Methods. Ten microliters of the final extract and 200 pmol of authentic DMT-NO in 10 μl of methanol were applied to the SCX column. The (a) excitation and (b) emission spectra of Metabolite D and authentic standard DMT-NO were then recorded.

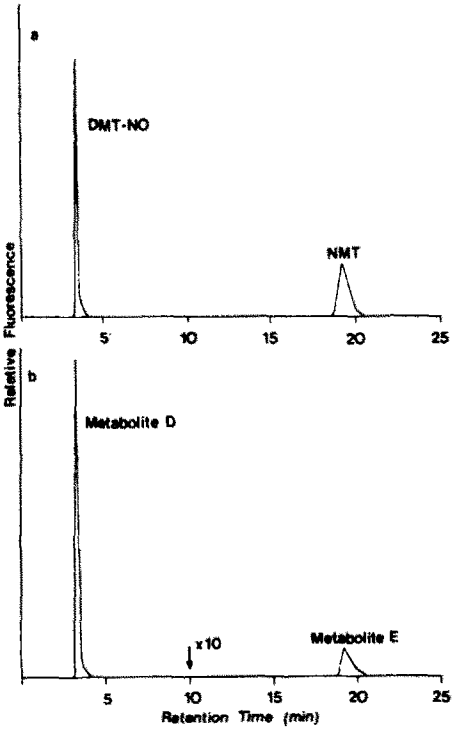

Fig. 4. Comparison of chromatography of Metabolites D and E formed in the presence of liver extracts with that of DMT-NO and NMT. A sample (100 μl) of those SCX column eluent fractions containing Metabolites D and E was applied to the silica column. Authentic samples of DMT-NO and NMT (500 pmol in 10 μl of methanol) were similarly chromatographed. Compounds were detected by fluorescence spectroscopy (λ_{ex} 279 nm, λ_{em} 348 nm). To facilitate the detection of Metabolite E, the sensitivity of detection was increased by factor of 10× at 10 min.

displayed retention time characteristics identical to those of DMT-NO (Fig. 4).

Of the remaining products, Metabolite E was identified as NMT on the basis of its retention time characteristics on both the SCX and silica columns. Because of its minor quantitative significance, no attempts to further characterise this metabolite were undertaken. Metabolite C, a minor product of DMT metabolism, remains unidentified.

Fractions of the SCX column eluent containing the substrate DMT were isolated, and the lyophilisate was subjected to secondary chromatography on the silica column under conditions previously shown to separate DMT and its potential metabolite MTHBC [15]. MTHBC was not detected in these fractions. Similar analyses of eluent fractions containing the metabolite NMT failed to reveal the presence of any co-eluting THBC.

Comparison of metabolism of DMT in various tissue extracts

Following the characterisation of the various products formed in the presence of liver tissue extracts, the metabolism of DMT in the presence of homogenates of other tissues, namely the kidney and the brain, was examined. Table 1 compares the relative rate of synthesis of individual metabolites of DMT in the presence of brain and kidney homogenates with that of the liver. Marked differences in the rates of synthesis of individual metabolites of DMT were observed in the presence of extracts from these various tissues.

Under the conditions described, oxidative deamination of DMT to IAC (which was further metabolised to IAA) appeared to be the major route of metabolism when DMT was incubated with homogenates prepared from either liver or brain tissue.

Table 1. Rate of formation of metabolites

Tissue	IAA*	IAC*	DMT-NO	NMT
	(pmol·min^{-1}·mg protein^{-1})			
Brain	72.7 ± 8.4 (4)	63.3 ± 9.4 (4)	1.7 ± 3.8 (3)	ND† (3)
Kidney	33.6 ± 1.5 (4)	24.3 ± 0.9 (4)	244.7 ± 19.8 (6)	ND (3)
Liver	283.1 ± 44.4 (4)	792.1 ± 87.7 (4)	585.1 ± 73.1 (6)	3.6 ± 4.0 (5)

Assays for metabolite formation were performed and the products were quantitated as described. Values are presented as means ± SEM for the number of determinations in parentheses.

* IAA and IAC were quantitated following secondary chromatography on the ODS column. Other quantitations were performed on the SCX column.

† Not detectable.

While DMT-NO was a prominent metabolite formed in the presence of kidney and liver homogenates, the N-oxidation of DMT remained only a very minor route of metabolism in the presence of brain tissue extracts. Whereas small quantities of NMT were formed in the presence of liver tissue, this metabolite was not detected following the incubation of DMT with either brain or kidney extracts.

During studies on the metabolism of DMT by liver, kidney and brain homogenates, the N-demethylation of DMT to tryptamine was not evident. The failure to detect this potential metabolite (retention time 5.2 min) and the apparently low rates of NMT synthesis catalysed by rat liver tissue extracts could be a consequence of their rapid metabolism by monoamine oxidase. It therefore was of interest that, despite a marked inhibition of the oxidative deamination of DMT in liver tissue extracts from animals pretreated witn iproniazid, the accumulation of tryptamine was not evident (Fig. 1b). In contrast to the observation of Barker *et al.* [14] that iproniazid pretreatment inhibited the synthesis of DMT-NO in the presence of brain homogenates by up to 95%, a similar inhibition of the synthesis of DMT-NO by liver homogenates was not evident in our studies.

Metabolism of 5MeODMT

A preliminary study was also conducted to demonstrate the application of the above techniques for the study of the metabolism of 5MeODMT by rat liver homogenates. Due to limitations on the availability of authentic standards, certain metabolites could not be extensively characterised. However, as Fig. 5 indicates, the pattern of metabolism of 5MeODMT appears to be similar to that already described for DMT. On the basis of the localisation of the enzymes involved in the synthesis of Metabolite A (and B), the inhibition of that synthesis in iproniazid-treated animals and a comparison of fluorescence spectroscopic and retention time characteristics on SCX and ODS columns with an authentic standard, Metabolite A was identified as 5MeOIAA. Also by analogy with the corresponding metabolite of DMT, Metabolite B was assigned the identity of 5-methoxyindoleacetaldehyde. In view of the similarities in the localisation of the enzymes involved in the synthesis of Metabolite D and its dependence on exogenous NADP(H), this product probably corresponds to the *N*-oxide of 5MeODMT.

As with DMT, the N-demethylation of 5MeODMT appears to be a relatively minor route of metabolism, and the accumulation of 5MeONMT (Metabolite E) was not facilitated by the inhibition of monoamine oxidase activity.

Only traces of 5OHDMT (Metabolite C) were detected following the incubation of 5MeODMT with liver homogenates, indicating that it is not a significant route of metabolism under the conditions examined. The further characterisation of the *N*-oxide and indoleacetaldehyde derivatives of 5MeODMT awaits the availability of authentic standards.

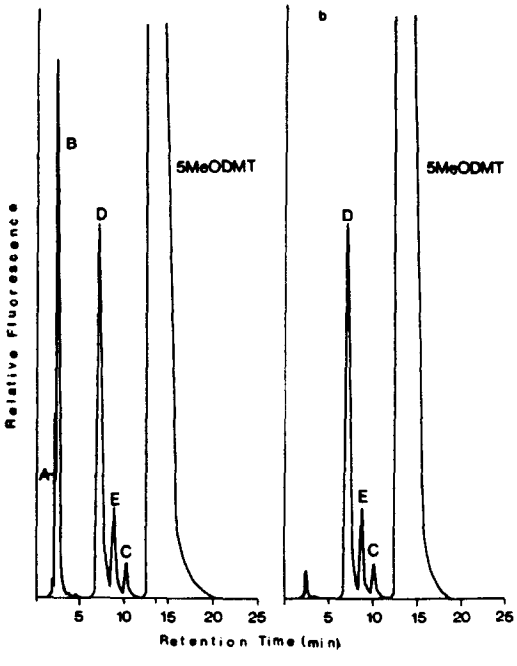

Fig. 5. Chromatograms of 5MeODMT and its metabolites formed (a) in the presence of rat liver homogenate, and (b) in the presence of liver homogenate from rats pretreated with iproniazid. 5MeODMT was incubated in the presence of rat liver homogenate. Ten microliters of the final extracts were applied to the SCX column. Compounds were detected by fluorescence spectroscopy (λ_{ex} 270 nm. λ_{em} 336 nm).

DISCUSSION

The LC-fluorometric methods we have developed [15, 17] have been used for the separation, quantitation, and characterisation of derivatives of DMT synthesised in the presence of rat tissue extracts. A number of products, including IAA, IAC, NMT and DMT-NO, have been identified as metabolites. A further metabolite (Metabolite C) remains unidentified.

The compatibility of the primary cation exchange chromatographic procedure with aqueous/alcoholic media and the utilization of detection techniques based on the natural fluorescence properties of the indole moiety have allowed the simultaneous assay of a number of metabolites of DMT without the need for the prior solvent extraction or the need for derivatisation. The non-destructive nature of fluorescence detection combined with the use of ammonium acetate based mobile phases ensured the efficient recovery of individual metabolites from the SCX column eluent for further characterisation. The identification of metabolites was supported by studies on localisation, cofactor requirements, and the effect of monoamine oxidase inhibitors on the enzymes involved in their synthesis.

Oxidative deamination by monoamine oxidase and N-oxidation by NADPH-dependent microsomal enzymes have been identified as the major routes of metabolism of DMT in liver and kidney tissues. In brain, only oxidative deamination emerged as a major metabolic route, the N-oxidation of DMT in brain remaining of very minor significance.

In contrast to oxidative deamination and N-oxidation, the N-demethylation of DMT in all tissues examined remains a very minor route of metabolism. 5MeODMT appears to be metabolised in liver tissue by mechanisms essentially analogous to those already described for DMT. The O-demethylation of 5MeODMT to 5OHDMT, previously described as a major route of metabolism of 5MeODMT *in vivo* in rats [9], did not emerge as a quantitatively significant pathway under the present conditions.

The identification of oxidative deamination as a major route of metabolism of DMT is consistent with the observation that pretreatment of rats with monoamine oxidase inhibitors markedly reduces the clearance of administered DMT from tissues such as brain and liver [18].

It has been debated whether the indoleacetic acid and indoleacetaldehyde formed from DMT are the result of direct oxidative deamination or the action of monoamine oxidase on intermediary metabolites such as NMT, DMT-NO or tryptamine [14, 19, 20]. Our failure to observe a marked increase in the levels of NMT and DMT-NO or the appearance of tryptamine in the presence of liver extracts from iproniazid-pretreated animals (despite evidence of a dramatic inhibition of the synthesis of indoleacetic acid and indoleacetaldehyde) supports the view that DMT is metabolised predominantly by direct oxidative deamination.

The inability to detect the tetrahydro-β-carbolines, THBC and MTHBC, following incubation of DMT with liver tissue extracts indicates that the synthesis of such metabolites does not represent a significant route of metabolism under the conditions used.

Studies are currently underway to correlate these observations with the metabolite profiles found in tissues and in body fluids following the administration of DMT and 5MeODMT *in vivo*.

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