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IN VIVO METABOLISM OF 5-METHOXY-*N*,*N*-DIMETHYLTRYPTAMINE AND *N*,*N*-DIMETHYLTRYPTAMINE IN THE RAT

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Abstract—Following intraperitoneal administration, 5-methoxy-*N*,*N*-dimethyltryptamine and *N*,*N*-dimethyltryptamine are subject to both a very rapid uptake into, and clearance from, all tissues examined. The current studies *in vivo* confirm previous *in vitro* observations that the routes involved in the metabolism of these compounds include oxidative deamination, N-demethylation, O-demethylation, and N-oxidation. The analysis of metabolic profiles in various tissues led to the identification of the *N*-oxides as major metabolites. The successful inhibition and redirection of metabolism away from the indole acids towards the parent compounds and their structurally unique metabolites were demonstrated in animals pretreated with iproniazid.

The psychotomimetic indolealkylamines, *N*,*N*-dimethyltryptamine (DMT), 5-methoxy-*N*,*N*-dimethyltryptamine (5MeODMT) and 5-hydroxy-*N*,*N*-dimethyltryptamine (5MeODMT), have now been detected in a number of human body fluids including urine, plasma and cerebrospinal fluid [1–5]. Attempts have been made to relate the concentration of these compounds to the presence of psychotic illnesses in humans [6–10].

Despite compelling evidence that the psychotomimetic indolealkylamines are both rapidly and extensively metabolized by mammalian tissues [10–14] prior to their excretion, little is known about the relative significance of individual routes of metabolism.

We recently studied [15] a number of potential routes of metabolism evident following the incubation of DMT and 5MeODMT with extracts prepared from various tissues of the rat. We now assess the significance of these routes *in vivo* based on an analysis of the metabolic profiles present in various tissues following the administration of both DMT and 5MeODMT.

MATERIALS AND METHODS

Materials. Iproniazid phosphate, 5-hydroxy-*N*,*N*-dimethyltryptamine (5OHDMT), *N*-methyltryptamine (NMT), *N*,*N*-dimethyltryptamine (DMT) and 5-methoxy-*N*,*N*-dimethyltryptamine (5MeODMT) were purchased from the Sigma Chemical Co. 5-Methoxy-*N*-methyltryptamine (5MeONMT) and *N*,*N*-dimethyltryptamine-*N*-oxide (DMT-NO) were donated by Dr. S. A. Barker, University of Alabama. 5-Methoxy-*N*,*N*-dimethyltryptamine-*N*-oxide (5MeODMT-NO) was synthesised by the method of Fish *et al.* [16]. All solvents used for chromatography were of analytical purity.

Instrumentation. Separation and quantitative analysis of the indolealkylamines and their metabolites were performed on a Perkin–Elmer Series 3B liquid chromatograph. All samples were injected on the column using a Rheodyne 7105 injector, fitted with a 175 μl loop.

The spectroscopic detectors that were used included a Perkin–Elmer 650-10S and a Perkin–Elmer 3000 fluorescence spectrometer.

Animals. Adult male Sprague–Dawley rats in the weight range of 250–350 g were subjected to a 12:12 hr dark:light cycle. Food and water were available to all animals *ad lib.*

Injection techniques. All drug administration to rats was via intraperitoneal injection. DMT and 5MeODMT were dissolved in 0.1 M HCl, and the pH was adjusted to 7 with 0.1 M NaOH. All other drugs were dissolved in 0.9% NaCl.

Monoamine oxidase inhibitor pretreatment. Individual adult male Sprague–Dawley rats were treated with iproniazid phosphate (100 mg/kg body weight) 3 hr prior to treatment with the indolealkylamine.

Preparation of tissue sample for analysis. Rats were divided into groups of four after treatment with DMT or 5MeODMT (10 mg/kg body weight). Groups were killed at 5, 10, 15, 30, 45 and 70 min after the administration of indolealkylamine alone. Control animals were treated with an equal volume of normal saline and killed after 10 min. In the case of the monoamine oxidase inhibitor pretreatment, animals were killed 45 min after the administration of DMT and 5MeODMT.

Rats were decapitated using a guillotine. The brain, liver, kidney and adrenal glands were rapidly removed and immediately frozen to −196° in liquid nitrogen. The blood was collected in heparinised tubes and cooled at 4° in an ice bath. This entire process was completed in less than 2 min.

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Assay for the formation of metabolites in tissues. With the exception of adrenal tissue, all tissues and blood samples were homogenized in 5 vol. of chilled 70% acetonitrile using a Polytron homogeniser (Kinematica, Switzerland). The adrenals were combined and homogenised in 1 ml of 70% acetonitrile. The homogenates were centrifuged for 15 min at 10,000 *g* and 4°. The supernatant fraction was removed and the pellets were rehomogenised in the same volume of acetonitrile and centrifuged. Aliquots of the combined supernatant fractions were analysed for the presence of the administered indolealkylamines and their metabolites on the SCX column using on-line fluorescence spectroscopy. Quantitation was achieved by comparison of relative fluorescence (peak height) with authentic standards, and assigned identities were confirmed by gas chromatography-mass spectrometry (unpublished observations).

Recoveries of DMT, 5MeODMT and their metabolites were determined by spiking tissues from control animals with 1.5 nmol of authentic standards prior to homogenisation. With the exception of the recovery of 5MeONMT and 5OHDMT from liver (55%), the efficiency of extraction of all other compounds from the various tissues was in the range of 70–100%, with coefficients of variation of less than 5%.

Separation of the indolealkylamines and their metabolites was achieved using a strong cation exchange column (Whatman Partisil 10 SCX 25 cm × 4.6 mm i.d. 10 μm), protected by a 3 cm × 2.8 mm precolumn packed with Whatman Co-Pell ODS. The mobile phase was methanol–0.083 M acetic acid/ammonia buffer (pH 4.4) (40:70) and was maintained at a flow rate of 1.5 ml/min. Conditions for fluorescence detection were λ_{ex} 280 nm and λ_{em} 357 nm in the case of DMT, and λ_{ex} 270 nm and λ_{em} 336 nm for 5MeODMT.

RESULTS

Analysis of the tissue concentrations of DMT and 5-MeODMT following administration indicate both a very rapid uptake and clearance of these compounds from all tissues examined. Maximum concentrations were achieved within 5 min in all tissues examined except brain where these occurred at about 10 min. This period of net uptake was followed by a rapid clearance, complete elimination being achieved within a period of 30–70 min. The estimated half-lives of elimination of DMT and 5-MeODMT from various tissues are presented in Table 1.

Our previous study [15] indicated that, following the incubation of DMT and 5MeODMT with tissues from the rat, metabolic pathways include monoamine oxidase catalysed oxidative deamination, N-demethylation, N-oxidation and, in the case of 5MeODMT, O-demethylation. Of the metabolites formed *in vitro*, which retained structural characteristics uniquely identifiable with those of the parent compound (i.e. were not potential metabolites of other indolealkylamines), the *N*-oxides appeared to be of the greatest quantitative significance [15].

Table 1. Estimated half-lives of elimination of DMT and 5MeODMT from rat tissues following their i.p. administration

Tissue	Estimated half-life (min) DMT	5MeODMT
Brain	5.7	6.0
Liver	9.6	13.6
Kidney	17.2	8.5
Adrenal	ND*	16.0
Blood	15.8	6.4

* Not determined.

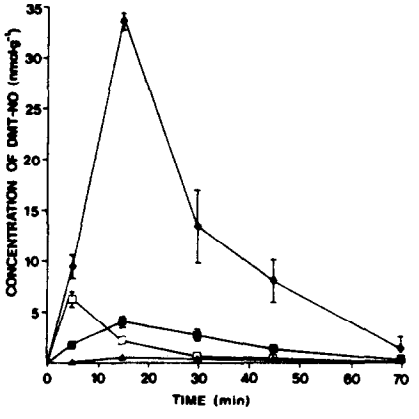

Fig. 1. Concentration of DMT-NO (nmoles/g wet tissue) in tissues following the i.p. administration of DMT. Rats were killed at specified intervals, and the levels of DMT-NO were determined in brain (▲), liver (□), kidney (◆) and blood (■). Results are expressed as mean ± SEM for four determinations.

The high concentrations of the *N*-oxides in both liver and kidney tissue at 10 min indicate the significance of this metabolic route *in vivo* (Figs. 1 and 2). The low concentrations of the *N*-oxides detected in brain correlate well with the low rates of DMT-NO formation observed in the presence of the brain tissue extracts *in vitro* [15].

Of the alternative metabolic routes observed in our *in vitro* studies [15], N-demethylation appeared to be of only minor quantitative significance. The very small quantities of NMT and 5MeONMT detected in the tissues (<6 nmol/g at all times) confirm the extremly minor nature of this route of metabolism.

Despite previous claims that O-demethylation of 5OHDMT represents a major route of metabolism of 5MeODMT in the rat [13], our studies [15] indicated that *in vitro* O-demethylation was, in fact, a minor pathway. In support of these latter observations, only low concentrations of 5OHDMT (<4 nmol/g at all times) were detected in liver, kidney and adrenal tissue following 5MeODMT administration, although the possible intervention of phase II metabolism, e.g. glucuronide conjugation [11], must be considered.

To confirm the major significance of oxidative deamination as a route of metabolism for DMT and 5MeODMT *in vivo* and to assess the feasibility of

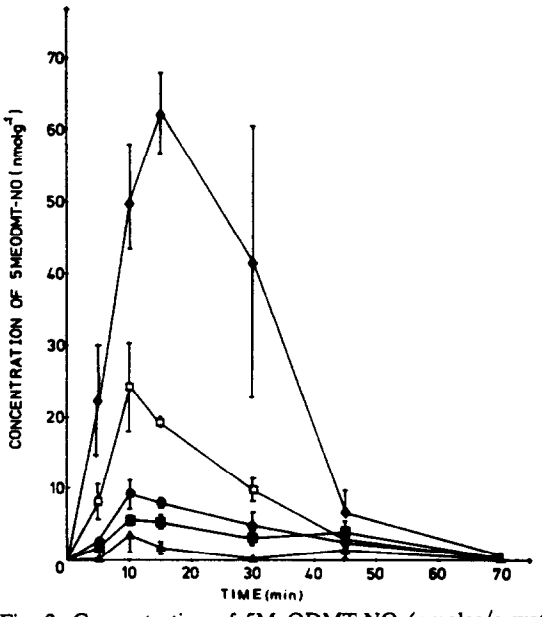

Fig. 2. Concentration of 5MeODMT-NO (nmoles/g wet tissue) in tissues following the i.p. administration of 5MeODMT. Rats were killed at specified intervals, and the levels of 5MeODMT-NO were determined in brain (▲), liver (□), kidney (◆), adrenals (●) and blood (■). Results are expressed as mean ± SEM for four determinations.

inhibiting and/or redirecting the metabolism of these compounds towards the more characteristic derivatives, the metabolic profiles were re-examined using animals pretreated with the monoamine oxidase inhibitor iproniazid. The effective elevation in concentrations of DMT and 5MeODMT remaining in the tissues 45 min after drug administration is consistent with the major significance of oxidative deamination (Tables 2 and 3).

More significantly, however, iproniazid pretreatment was also accompanied by increases in the tissue concentration of other characteristic metabolites. The increase in DMT-NO concentrations remaining in the liver was particularly striking. Despite the statistical variation, increases in the concentrations

Table 2. Effect of iproniazid pretreatment on the levels of DMT and metabolites in rat tissues 45 min after DMT administration

Tissue	Concentration in tissues (nmol/g wet tissue) DMT	DMT-NO	NMT
Brain			
Untreated	5.6 ± 0.7	0.24 ± 0.04	ND*
Treated	26.5 ± 5.9†	0.9 ± 0.4	0.7 ± 0.3
Liver			
Untreated	4.6 ± 0.7	0.19 ± 0.04	ND
Treated	37.1 ± 5.1‡	33.7 ± 3.0‡	3.9 ± 0.9
Kidney			
Untreated	19.5 ± 2.0	8.9 ± 4.2	ND
Treated	56.4 ± 9.8†	29.9 ± 10.7	7.2 ± 1.3
Blood			
Untreated	1.1 ± 0.1	1.6 ± 0.1	ND
Treated	3.3 ± 0.6†	5.0 ± 1.1†	0.4 ± 0.2

The concentrations of DMT and its characteristic metabolites were determined 45 min after drug administration to control and iproniazid-pretreated animals. Results are presented as mean ± SEM for determinations on three to four animals.

* Not detectable.

†‡ Significantly different from untreated control (two-tail Student's *t*-test): † $P < 0.01$ and ‡ $P < 0.001$.

Table 3. Effect of iproniazid pretreatment on the levels of 5MeODMT and metabolites in rat tissues 45 min after 5MeODMT administration

Tissue	Concentration in tissues (nmol/g wet tissue) 5MeODMT	5MeODMT-NO	5MeONMT	5OHDMT
Brain				
Untreated	0.08 ± 0.01	0.37 ± 0.24	0.003 ± 0.003	ND*
Treated	7.8 ± 1.9†	0.51 ± 0.03	0.03 ± 0.02†	ND
Liver				
Untreated	1.2 ± 0.1	0.70 ± 0.19	0.09 ± 0.02	0.07 ± 0.01
Treated	13.6 ± 0.7‡	7.2 ± 3.7	1.6 ± 0.4†	0.03 ± 0.03
Kidney				
Untreated	0.7 ± 0.02	1.6 ± 0.7	0.27 ± 0.24	ND
Treated	14.1 ± 3.9†	41.2 ± 17.7	0.5 ± 0.5	0.06 ± 0.06
Blood				
Untreated	0.03 ± 0.01	0.91 ± 0.35	ND	0.01
Treated	1.2 ± 0.4†	3.4 ± 1.4	0.17 ± 0.17	ND
Adrenal				
Untreated	1.7 ± 0.1	0.60 ± 0.17	0.19 ± 0.04	0.26 ± 0.07
Treated	16.5 ± 2.8§	8.6 ± 1.8†	1.65 ± 0.24†	0.58 ± 0.12

The concentrations of 5MeODMT and its characteristic metabolites were determined 45 min after drug administration to control and iproniazid-pretreated animals. Analyses were performed as described in Materials and Methods. Results are presented as mean ± SEM for determinations on three to four animals.

* Not detectable.

†–§ Significantly different from untreated control (two-tail Student's *t*-test): † $p < 0.05$, ‡ $p < 0.001$, and § $p < 0.01$.

of the *N*-oxides of DMT and 5MeODMT were also clearly evident in kidney, adrenal and liver tissue. In contrast to these peripheral tissues, the low concentrations of the *N*-oxides observed in the brain and blood of control animals were not elevated greatly by iproniazid pretreatment.

The concentration of 5MeONMT detected in the adrenals at 45 min was also elevated significantly by iproniazid pretreatment. Also, NMT, previously undetectable at 45 min in the tissues of animals injected with DMT, was readily identified in liver, adrenal and kidney tissue. While these studies indicated that the very low concentrations of 5MeONMT and NMT detected in the tissues of control animals may, in part, be due to their further metabolism by monoamine oxidase, the limited absolute increases following monoamine oxidase inhibition confirm the relatively minor significance of N-demethylation in the overall metabolism of the psychotomimetic indolealkylamines.

Following the inhibition of monoamine oxidase activity, the O-demethylation of 5MeODMT did not appear to be similarly affected.

DISCUSSION

Intraperitoneal administration of 5MeODMT and DMT was followed by their rapid redistribution into kidney, liver and brain. Both drugs were detected in brain as early as 5 min after their administration. These observations are consistent with the rapid onset of the behavioural and psychotomimetic effects of these drugs in experimental animals and humans [17]. The high rates of clearance from all tissues including brain explain the short duration of the psychotomimetic effects induced by these drugs [17].

Despite their extremely rapid clearance from tissues, it has been reported that less than 0.2% of an administered dose of DMT to humans is recovered unmetabolised in urine. Similarly, less than 0.1% of an injected dose of 5MeODMT was detected ultimately in the urine of rats [13, 14, 18].

The postulate that the rapid clearance of these compounds *in vivo* is the result of metabolism is supported by both the detection of appropriate metabolites *in vivo* and our observations that iproniazid-mediated inhibition of oxidative deamination (identified as a major route of metabolism of 5MeODMT and DMT *in vitro*) slowed the clearance of the administered drugs from all tissues examined.

Microsomal enzymes that are responsible for the formation of the characteristic *N*-oxide metabolites *in vitro* have not appeared to play a significant role in the metabolism of the psychotomimetic indolealkylamines *in vivo* [19]. In contradiction, we have now demonstrated that N-oxidation constitutes a major route of metabolism of 5MeODMT and DMT.

While due regard must be given to possible species differences, the work described in this report indicates that any future clinical studies in humans should involve an analysis not only of 5MeODMT and DMT but also of their characteristic metabolites, e.g. the *N*-oxides. In addition, the use of monoamine oxidase pretreatment should be considered as a means of inhibiting and/or redirecting metabolism away from the non-characteristic indole acids, towards these more characteristic metabolites.

The rapidity of clearance of not only the parent compounds but also their characteristic metabolites from all tissues examined may be indicative of a high rate of renal excretion for all these compounds. The urinary excretion of 5MeODMT and DMT and their characteristic metabolites and the influence of monoamine oxidase inhibition on the metabolic profile in urine are, therefore, currently being examined.

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