Biochemical Pharmacology, Vol. 36, No. 13, pp. 2235–2237, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
© 1987. Pergamon Journals Ltd.

Urinary excretion of 5-methoxy-*N*,*N*-dimethyltryptamine, *N*,*N*-dimethyltryptamine and their *N*-oxides in the rat

(*Received* 11 *September* 1986; *accepted* 16 *December* 1986)

The presence of the psychotomimetic indolealkylamines 5-methoxy-*N*,*N*-dimethyltryptamine (5MeODMT), *N*,*N*-dimethyltryptamine (DMT) and 5-hydroxy-*N*,*N*-dimethyltryptamine (5OHDMT) in a number of human body fluids including urine, plasma and cerebrospinal fluid has been widely documented [1–5]. Attempts have been made to relate the concentrations of these compounds, particularly in urine, to the presence of psychotic illness in man [6–9]. A substantial body of evidence has suggested that the rapid clearance of these compounds from mammalian tissues, observed immediately following administration, is the result of extraordinarily rapid and extensive metabolism [10–13].

In recent studies on the rat [10, 14], we identified a number of routes of metabolism for DMT and 5MeODMT. Of the observed metabolites, the *N*-oxides of DMT and 5MeODMT emerged as the metabolites of greatest quantitative significance which retained structural characteristics identifiable with the parent compounds [10, 14]. Pretreatment with the monoamine oxidase inhibitor iproniazid proved a successful strategy for the re-direction of the metabolism of DMT and 5MeODMT towards such characteristic metabolites *in vivo*. During the course of these studies, it became evident that not only DMT and 5MeODMT but also their *N*-oxides were subject to rapid clearance from the tissues. We now examine the urinary excretion of administered DMT, 5MeODMT and their *N*-oxides and the influence of pretreatment with monoamine oxidase inhibitors on their cumulative concentrations in urine.

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 synthesized by the method of Fish *et al.* [15].

All solvents used for chromatography were of analytical purity.

Instrumentation. Separation and quantitative analyses 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 250–350 g were subjected to a 12:12 hr dark:light cycle, and 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 wt) 3 hr prior to treatment with the indolealkylamine or pargyline hydrochloride (75 mg/kg body wt) at 24 hr and again 2 hr prior to indolealkylamine treatment.

Collection of urine specimens for analysis. Individual adult male Sprague–Dawley rats were treated with either DMT or 5MeODMT (10 mg/kg body wt). Control animals were injected with an equal volume of normal saline. Immediately following drug administration, the rats were placed in individual metabolic cages where they were fed and watered *ad lib.* Urine specimens were collected in tubes maintained at 0° and protected from light, at intervals over a 24-hr period. Samples were stored at −20° for a maximum of 20 hr prior to analysis. Studies had indicated that in urine the indolealkylamines and their metabolites were stable for at least 24 hr when stored under these conditions (less than 1% degradation).

Analysis of DMT, 5MeODMT and their metabolites in urine. To an aliquot of urine was added an equal volume of 70% acetonitrile. Following vortexing and centrifugation at 3000 *g* for 10 min, samples of the supernatant fraction were analysed for the presence of the administered indolealkylamines and their metabolites. The recoveries of all metabolites ranged from 97 to 100%.

Separation and analysis of the indolealkylamines and their metabolites were achieved using a strong cation exchange column (Whatman Partisil 10 SCX 25 cm × 4.6 mm i.d.) protected by a 3 cm × 2.8 mm precolumn packed with Whatman CoPell ODS. The mobile phase was methanol:0.083 M acetic acid/ammonia buffer, pH 4.4 (30:70) at a flow rate of 1.5 ml/min [14].

Results

Levels of 5MeODMT and DMT appearing in rat urine following 5-MeODMT administration. The levels of 5MeODMT and DMT were determined in rat urine during the 24 hr following i.p. administration. Following their administration, 5MeODMT and DMT appeared rapidly in the urine, with at least 90% of the total amount excreted in 24 hr appearing within 3.5 hr. Less than 4% of the total excreted appeared after the initial 12-hr period. While the rate of appearance of 5MeODMT and DMT in the urine paralleled their rapid disappearance from the tissues, the fact that less than 0.2 and 1.1% of the administered doses, respectively, could ultimately be recovered from urine as the parent compound (Table 1) is indicative of extensive metabolism prior to excretion.

Of the structurally characteristic metabolites detected in rat tissues immediately following 5MeODMT and DMT administration, 5MeODMT-NO and DMT-NO appear to be the metabolites of major quantitative significance [10]. An examination of changes in the metabolic profile in tissues such as kidney and liver with time had indicated that, like 5MeODMT and DMT themselves, their *N*-oxides were also subject to a rapid clearance from these tissues. The rapid rate of appearance of 5MeODMT-NO and DMT-NO in the urine (over 90% of the 5MeODMT-NO and DMT-NO excreted in 24 hr appeared during the first 3 hr following drug administration) suggests this is a consequence of their rapid renal excretion (Figs. 1 and 2).

Although an analysis of metabolic profiles in various rat tissues following 5MeODMT and DMT administration suggested that, in contrast to N-oxidation, N-demethylation

Table 1. Effect of iproniazid pretreatment on the urinary excretion of 5MeODMT and its metabolites

	5MeODMT		5MeODMT-NO		5MeONMT		5OHDMT	
Pretreatment	nmol excreted	% of Dose given	nmol excreted	% of Dose given	nmol excreted	% of Dose given	nmol excreted	% of Dose given
Control	18.9 ± 3.6	0.14 ± 0.02	984 ± 177	7.4 ± 1.0	ND*	ND	13.9 ± 1.1	0.11 ± 0.02
Iproniazid	470 ± 57	3.0 ± 0.03†	7468 ± 162	48.8 ± 1.0‡	100.1 ± 2.1	0.50 ± 0.15†	30.6 ± 5.1	0.23 ± 0.03

Shown are the amounts of 5MeODMT and its characteristic metabolites excreted in the urine during the 24 hr following 5MeODMT administration. Results are the mean ± SEM for determinations on three animals.

* Not detectable.

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

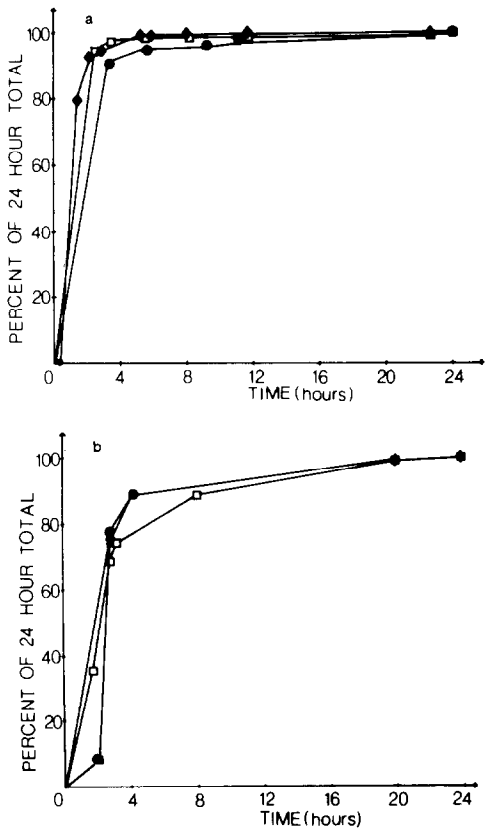

Fig. 1. Amount of 5MeODMT-NO appearing in the urine during the 24 hr following 5MeODMT administration to: (a) control animals, and (b) animals pretreated with iproniazid.

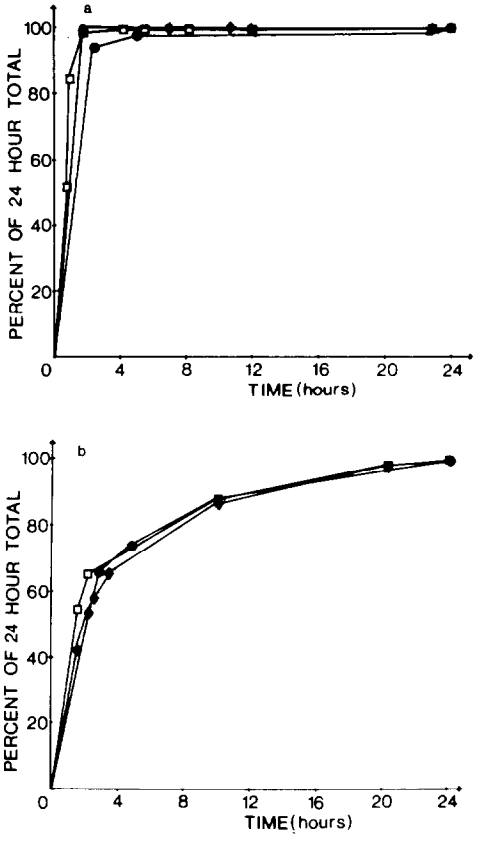

Fig. 2. Amount of DMT-NO appearing in the urine during the 24 hr following DMT administration to: (a) control animals, and (b) animals pretreated with iproniazid.

and O-demethylation represent very minor routes of metabolism [10], the failure to detect significant quantities of these metabolites in tissues could alternatively be the result of an unusually rapid renal clearance. The failure to detect 5MeONMT and the minor quantities of NMT and 5OHDMT appearing in urine (Table 1) do not support the latter hypothesis.

Effect of MAO inhibition on the urinary excretion of 5MeODMT and DMT and their N*-oxides.* Our previous *in vitro* and *in vivo* studies [10, 14] have indicated that, in addition to the metabolic routes leading to these characteristic metabolites, a major alternative route catalysed by monoamine oxidase leads to the formation of the non-characteristic indoleacids. Analyses of the metabolic profiles in the tissues of animals pretreated with iproniazid have revealed that monoamine oxidase inhibitors (MAOI) may facilitate the redirection of the metabolism of both 5MeODMT and DMT towards these structurally characteristic compounds [10].

In the present study, marked changes in the levels of the parent compound and its metabolites excreted in urine were also evident following MAOI pretreatment. The concentrations of 5MeODMT, DMT and their *N*-oxides appearing in the urine were increased markedly.

Despite the appearance of increased urinary 5MeONMT and NMT following iproniazid pretreatment, these metabolites ultimately accounted for only 0.6% of the administered dose.

Tables 1 and 2 present a summary of the total amounts of 5MeODMT, DMT and a number of their structurally

Table 2. Effect of MAO inhibitor pretreatment on the excretion of DMT and its metabolites

Pretreatment	DMT		DMT-NO		NMT	
	nmol excreted	% of Dose given	nmol excreted	% of Dose given	nmol excreted	% of Dose given
Control	191.3 ± 33.7	1.1 ± 0.1	1084 ± 389	6.5 ± 2.3	4.7 ± 4.2	0.02 ± 0.02
Iproniazid	360 ± 82	2.1 ± 0.4	3539 ± 170	20.6 ± 0.5*	114 ± 17	0.6 ± 0.1*
Pargyline	376 ± 87.9	2.4 ± 0.7	2815 ± 549	17.7 ± 3.8	ND†	ND

Shown are the amounts of DMT and its characteristic metabolites excreted in the urine during the 24 hr following DMT administration. Results are the mean ± SEM for determinations on three animals.
* Significantly different from control (two-tailed Student's *t*-test): $P < 0.005$.
† Not detectable.

characteristic metabolites excreted in urine during the 24 hr following administration to control rats and to rats pretreated with iproniazid. These values are also expressed as a percentage of the total amount injected. The amount of the administered dose accounted for in urine as the parent compound and these structurally characteristic metabolites was greatly enhanced in animals pretreated with iproniazid, increasing from less than 8 to 53% and 24% of the administered dose of 5MeODMT and DMT respectively. Pretreatment with pargyline, an irreversible inhibitor of monoamine oxidase, did not induce further increases in the proportion of the administered dose (of DMT) appearing in urine as these characteristic metabolites.

Discussion

Analyses of urine immediately following the administration of DMT and 5MeODMT confirm the view that these compounds are subjected to rapid and extensive metabolism prior to excretion. Less than 0.2% of the administered dose of 5MeODMT and less than 1.5% of the dose of DMT appeared in urine unmetabolised. The fact that more than 90% of the total amount of DMT, 5MeODMT and their *N*-oxides excreted in 24 hr appeared in urine within 3.5 hr indicates that not only the parent compounds but also their *N*-oxides are subject to very rapid renal excretion.

Our studies *in vitro* [14] have indicated that a major route of metabolism involves the MAO catalysed oxidative deamination of DMT and 5MeODMT to their non-characteristic indoleacids. Analyses of the metabolic profiles in various tissues have indicated that pretreatment with the MAO inhibitor iproniazid may successfully be used to inhibit MAO and redirect metabolism towards structurally characteristic metabolites [10].

In support of this hypothesis, an examination of the metabolic profiles in urine revealed marked increases in the amounts of characteristic metabolites (in particular the *N*-oxides) excreted in animals pretreated with monoamine oxidase inhibitors. To date clinical studies attempting to relate the concentration of the psychotomimetic indolealkylamines in human body fluid to psychotic illnesses [1–9] have focused exclusively on an analysis of the parent compounds. The present studies indicate that the design of future clinical studies must take into account the extraordinary rapid metabolism and renal excretion of these compounds.

While the *N*-oxides of DMT and 5MeODMT (particularly following monoamine oxide inhibition) have emerged as characteristic metabolites of major quantitative significance, further studies may identify other metabolites of potential value in the analysis of the psychotomimetic indolealkylamines.

In summary, following their intraperitoneal administration, 5-methoxy-*N*,*N*-dimethyltryptamine and *N*,*N*-dimethyltryptamine were excreted rapidly in the urine. Despite the high rates of excretion, only a very small percentage of the administered dose appeared in the urine unmetabolised. An examination of the metabolic profile in urine led to the identification of a number of characteristic metabolites. Of these, the *N*-oxides were of the greatest quantitative significance. The excretion profiles of the *N*-oxides indicated that these metabolites were also subject to rapid excretion. Pretreatment of animals with the monoamine oxidase inhibitors resulted in a marked increase in the percentage of the administered dose appearing in urine as either the parent compounds or their *N*-oxides.

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

BALVANT R. SITARAM*
LYNN LOCKETT
GRAEME L. BLACKMAN
WILLIAM R. MCLEOD

* 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.

REFERENCES

1. M. Raisanen and J. Karkkainen, *J. Chromat.* **162**, 579 (1979).
2. M. C. H. Oon and R. Rodnight, *Biochem. Med.* **18**, 410 (1977).
3. B. R. Sitaram, G. L. Blackman, W. R. McLeod and G. N. Vaughan, *Analyt. Biochem.* **128**, 11 (1983).
4. J. R. Smythies, R. D. Morin and G. B. Brown, *Biol. Psychiat.* **14**, 549 (1979).
5. B. Angrist, S. Gershon, G. Sathananthan, R. W. Walker, B. Lopez-Ramos, L. R. Mandel and W. J. A. Van Denheuvel, *Psychopharmacologia* **47**, 29 (1976).
6. M. C. H. Oon, R. M. Murray, R. Rodnight, M. P. Murphy and J. L. Birley, *Psychopharmacology* **54**, 171 (1977).
7. M. Raisanen, M. Virkkunen, M. O. Huttunen, B. Furman and J. Karkkainen, *Lancet* 700 (1984).
8. A. C. Cottrell, M. F. McLeod and W. R. McLeod, *Am. J. Psychiat.* **134**, 322 (1977).
9. R. Uebelhack, L. Franke and K. Seidel, *Biomed. Biochim. Acta* **42**, 1343 (1983).
10. B. R. Sitaram, L. Lockett, R. Talomsin, G. L. Blackman and W. R. McLeod, *Biochem. Pharmac.* **36**, 1509 (1987).
11. J. Kaplan, L. R. Mandel, R. Stillman, R. W. Walker, W. J. A. Van Denheuvel, J. C. Gillin and R. J. Wyatt, *Psychopharmacologia* **38**, 239 (1974).
12. S. Agurell, B. Holmstedt and J. E. Lindgren, *Biochem. Pharmac.* **18**, 2771 (1969).
13. E. Sanders-Bush, J. A. Oates and M. T. Bush, *Life Sci.* **19**, 1407 (1976).
14. B. R. Sitaram, R. Talomsin, G. L. Blackman and W. R. McLeod, *Biochem. Pharmac.* **36**, 1503 (1987).
15. M. S. Fish, N. M. Johnson and E. C. Horning, *J. Am. chem. Soc.* **77**, 5892 (1955).