Biochemical Pharmacology, 1966, Vol. 15, pp. 2097–2103. Pergamon Press Ltd., Printed in Great Britain.

THE METABOLISM OF α-^{14}C-3,4-DIMETHOXYPHENETHYLAMINE

JACK W. SCHWEITZER and ARNOLD J. FRIEDHOFF

Department of Psychiatry and Neurology, New York University, New York, N.Y., U.S.A.

(*Received* 8 *June* 1966; *accepted* 6 *July* 1966)

Abstract—α-^{14}C-Dimethoxyphenethylamine hydrochloride was administered to rats, i.p., and the metabolites were isolated and identified from the 0–2·5 hr urine. The following compounds were positively identified (percentages of urinary ^{14}C are given in parentheses): dimethoxyphenylacetic acid (77), unchanged amine (15·5), and N-acetyl-3-methoxytyramine glucuronide (6·0). Tentative identification was also made for N-acetyl-3-methoxytyramine (ca. 0·2) and for trace amounts of N-acetyldimethoxyphenethylamine, 3-methoxy-4-hydroxyphenethanol glucuronide, and dimethoxyphenethanol. Pretreatment of rats with aldehyde and amine oxidase inhibitors resulted in anticipated increases or decreases in the excretion of some of the metabolites.

One of the metabolites, N-acetyldimethoxyphenethylamine, was found to be physiologically active, producing hypokinesis within 5 min. Administration of the ^{14}C-labeled compound resulted in the excretion of N-acetyl-3-methoxytyramine glucuronide only. These results, in conjunction with the failure to detect homovanillic acid and methoxytyramine after administering ^{14}C-dimethoxyphenethylamine, indicate that N-acetylation precedes O-demethylation.

3,4-DIMETHOXYPHENETHYLAMINE*[1–6] and the corresponding acid, DMPAA[7] have been detected in the urine of psychotic patients. The significance of these findings is not yet clear, since other investigators have also reported the presence of DMPEA in the urine of normal individuals[3] and in patients with Parkinson's disease,[8] and others have failed to find the compound.[9–12] These discordant findings may result in part from the manner in which DMPEA is metabolized in man. Thus psychotic patients, receiving 500 mg of DMPEA hydrochloride orally, excreted an average of 0·4 per cent as the unchanged amine within 24 hr, the major amount having been converted to DMPAA.[13] Clearly then, the excreted DMPEA appears to represent only a small fraction of the daily turnover of this compound. It is possible that DMPEA, if formed endogenously, would be metabolized to an even greater extent. Therefore it is important to identify all the significant metabolites of DMPEA so that meaningful comparisons of the daily turnover of this compound can be made. The present study was undertaken to investigate the metabolic fate of DMPEA in rats.

MATERIALS

α-^{14}C-DMPEA hydrochloride was synthesized from dimethoxybenzyliodide and

* Abbreviations used: DMPEA: 3,4-dimethoxyphenethylamine; DMPAA: 3,4-dimethoxyphenylacetic acid; NAMT: N-acetyl-3-methoxy-4-hydroxyphenethylamine; MHPE: 3-methoxy-4-hydroxyphenethanol; DMPE: 3,4-dimethoxyphenethanol; NPMT: N-propionyl-3-methoxy-4-hydroxyphenethylamine.

$Na^{14}CN$, as reported earlier.[14] Unlabeled DMPEA hydrochloride was prepared from the free base and HCl gas in ether and was recrystallized twice from benzene–chloroform. Acetyl (and propionyl) derivatives were prepared by treating the free bases in pyridine with acetic (or propionic) anhydride and recrystallizing with ether–heptane. Phenolic amides were obtained as water solutions by hydrolysis of the diacetyl or dipropionyl derivatives in 1 N HCl at 60° for 1 min. MHPE was synthesized by reduction of homovanillic acid and was recrystallized with heptane. DMPE was synthesized from diazotized DMPEA by hydrolysis with aqueous ethanol[15] and was recrystallized with heptane. DMPAA was purified by sublimation. β-Glucuronidase, with an activity of 1500 Fishman units/mg, was purchased from General Biochemicals.

METHODS

Groups of 100-g Wistar rats were injected per kg (i.p.) with 4 mg α-^{14}C-DMPEA hydrochloride (0·72 mc/m-mole) in 0·5 ml saline solution. Other groups received various drugs (Table 6) one or more hours prior to the injection of the labeled amine. Urine was collected for a period of 2·5 hr and 50–100 μl of the urine of each group was chromatographed on 1·5-inch wide strips of Whatman 1 paper in each of two solvent systems: *n*-butanol:acetic acid:water, 4:1:1 (solvent A) and ethanol:conc. ammonia:water, 16:1:3 (solvent B). Each chromatogram was scanned for ^{14}C with a Packard model 7201 paper scanner; then a 1·5-cm strip was cut away, divided into 1-cm sections from the origin to the front, and each section counted with a Tracerlab planchet counter. In this manner the ^{14}C contribution of each metabolite to the total ^{14}C excreted could be determined. For purposes of identification, the metabolites were isolated from the urine by chloroform extraction and back-extraction procedures at pH values dictated by the acidic properties of the metabolites. When necessary, the ^{14}C content of these fractions was determined with a Beckman scintillation counter, 90 per cent efficient for ^{14}C. Unextractable ^{14}C was also investigated. Formal identification of the metabolites was established by a comparison of solubility characteristics under varying conditions of pH, by co-chromatography and by co-crystallization with authentic compounds, as described below.

EXPERIMENTAL AND RESULTS

Paper chromatography of a portion of the urine of rats, 2·5 hr after injecting α-^{14}C-DMPEA, revealed three significant peaks in solvents A or B (see Methods).

TABLE 1. IDENTIFICATION OF THE ACIDIC METABOLITE AS DIMETHOXYPHENYLACETIC ACID

Co-chromatography with DMPAA		Co-crystallization with DMPAA in hexane solvent	
Solvent system	R_f of Acidic ^{14}C or DMPAA	Cryst. #	(counts/min/mg × 10^{-5})
Solvent A	0·90	1	2·14
Solvent B	0·67	2	2·14
Solvent B, i-propanol replacing ethanol	0·57	3	2·07
Solvent B, n-butanol replacing ethanol*	0·31	4	2·18

* Upper phase.

The major metabolite, accounting for 77 per cent of the urinary ^{14}C, was acidic. It was isolated by chloroform extraction of acidified urine and separated from neutral metabolites by back-extracting with bicarbonate solution. By co-chromatography in four solvent systems and by co-crystallization, this metabolite cannot be distinguished from DMPAA (Table 1). Homovanillic acid could not be detected, even in trace amounts.

The second most prominent peak, an amine representing about 15 per cent of the urinary ^{14}C, was extracted from the urine at pH 10 (after removing acidic and neutral substances at lower pH). The results in Table 2 indicate that this compound is

TABLE 2. IDENTIFICATION OF THE BASIC METABOLITE AS DIMETHOXYPHENETHYLAMINE

Co-chromatography with DMPEA		Co-crystallization with DMPEA hydrochloride using benzene—$CHCl_3$	
Solvent system	R_f of Basic ^{14}C or DMPEA	Cryst. #	(counts/min/mg)
Solvent A	0·75	1	2420
Solvent B	0·95	2	2560
Solvent A, i-propanol replacing butanol	0·85	3	2360
		4	2540
Butanol: formic acid: water, 12:1:6*	0·62		

* Upper phase

α-^{14}C-DMPEA. 3-Methoxytyramine, easily separable from DMPEA in several solvent systems, could not be detected.

The third peak, at about R_f 0·35 in solvent A or B, represents material that could not be extracted from urine with chloroform, regardless of pH. When either the labeled material, eluted from a chromatogram, or the residual urinary ^{14}C, was treated with β-glucuronidase at pH 4·5, all the ^{14}C was converted to material extractable up to pH 11. The new substance, subjected to 1 N HCl hydrolysis at 100°, yielded a compound extractable with chloroform only between pH values of 8 and 11. The original material, after enzymatic hydrolysis, is co-chromatographic with N-acetyl-3-methoxytyramine (NAMT) in two solvent systems (Table 3). Attempts to prepare authentic crystalline NAMT were only partially successful, whereas

TABLE 3. IDENTIFICATION OF THE UNEXTRACTABLE METABOLITE, AFTER ENZYMATIC HYDROLYSIS, AS N-ACETYL-3-METHOXY-4-HYDROXYPHENETHYLAMINE

Solvent system	System*	Metabolite	NAMT	MHPE	NPMT
Benzene: methanol: acetic acid, 45:8:4	TLC	0·42	0·42	0·50	
Chloroform	ITLC	0·38	0·38	0·67	0·63

* TLC: Glass plates coated 80 μ thick with Merck silica gel G. ITLC: Gelman Instrument Co. fibreglass strips impregnated with silica gel.

N,O-diacetylmethoxytyramine was easily crystallizable. Therefore the unconjugated metabolite was acetylated and the product was co-crystallized with diacetylmethoxytyramine (Table 4). In addition, it was demonstrated that the unconjugated metabolite did not co-chromatograph with MHPE or with NPMT, its closest homologue (Table 3).

TABLE 4. CO-CRYSTALLIZATION OF THE ENZYMATICALLY HYDROLYZED UNEXTRACTABLE METABOLITE, AFTER ACETYLATION, AND N,O-DIACETYL-3-METHOXYTYRAMINE

Crystallization #	(counts/min/mg)
1	125
2	124
3	124
4	119

Not observable on paper chromatograms is a mixture of neutral compounds that runs near the front in either solvent system. This fraction, approximately 0·5 per cent of the excreted ^{14}C, remains in the chloroform when the urine is extracted at low pH and then back-extracted with bicarbonate solution to remove ^{14}C-DMPAA. It can be further subdivided into a completely neutral portion and a phenolic fraction by extracting the chloroform with carbonate solution. The phenolic portion is composed exclusively of ^{14}C-NAMT, as demonstrated by co-chromatography with authentic material in two solvent systems. The substances remaining in the chloroform can be separated into four compounds by ILTC chromatography (see footnote to Table 3) in benzene:butanol, 100:2. One of these peaks co-chromatographed with N-acetyl-DMPEA. Moreover, when the entire extract was co-crystallized with N-acetylDMPEA, an initial fall in specific activity was noted, but after two recrystallizations, constant values were obtained. Another compound in this fraction was easily hydrolyzed to ^{14}C-DMPAA. A small peak that co-chromatographed with DMPE was also present, but no further characterization of this constituent has been made. A summary of the identified metabolites appears in Table 5.

When rats were pretreated with calcium carbimide prior to the administration of α-^{14}C-DMPEA, three incompletely separated peaks were found in the "glucuronide"

TABLE 5. URINARY METABOLITES OF DIMETHOXYPHENETHYLAMINE

Metabolite	% of Excreted ^{14}C*
Dimethoxyphenylacetic acid	77·0
Dimethoxyphenethylamine	15·5
N-Acetylmethoxytyramine glucuronide	6·0
N-Acetylmethoxytyramine	ca. 0·2
N-Acetyldimethoxyphenethylamine†	trace
3-Methoxy-4-hydroxyphenethanol glucuronide†	trace
Dimethoxyphenethanol†	trace

* The average of four experiments. Urine, collected for 2·5 hr after injecting α-^{14}C-DMPEA hydrochloride at 4 mg/kg, i.p. to rats, contained an average of 67·4 per cent of the administered ^{14}C.

† Tentatively identified.

region upon chromatography in solvent A. After treatment with β-glucuronidase, thin-layer chromatography (TLC and ILTC systems of Table 3) with co-chromatographed standards revealed that 53 per cent of the glucuronide fraction was composed of NAMT, 8 per cent was contributed by MHPE, and the remainder was accounted for by a compound that was less polar than the other two substances. Since calcium carbimide is an aldehyde oxidase inhibitor, the new peak may be 3-methoxy-4-hydroxyphenylacetaldehyde.

The glucuronide fraction of the urine of rats pretreated with Catron (1-phenyl-2-hydrazinopropane, a monoamine oxidase inhibitor) contained only ^{14}C-NAMT glucuronide.

The influence of various drugs on the metabolic excretion pattern of administered α-^{14}C-DMPEA was also investigated. The results are summarized in Table 6. Catron, as expected, greatly diminished the formation of DMPAA. As a consequence, more DMPEA was converted to NAMT glucuronide. Calcium carbimide decreased the DMPAA excretion somewhat, the difference appearing as MHPE and an unidentified metabolite, perhaps the phenolic aldehyde. A diminished excretion of conjugated NAMT was noted after treatment with mescaline or imipramine. Amphetamine and tolbutamide, the latter influencing the extent of catecholamine demethylation in the guinea pig,[16] did not change the ^{14}C excretion pattern significantly.

TABLE 6. DRUG EFFECTS ON THE METABOLIC EXCRETION PATTERN OF α-^{14}C-DMPEA

Drug	Dose (mg/kg)	Percentage of excreted ^{14}C*					
		DMPAA		DMPEA		NAMT glucuronide	
		A	B	A	B	A	B
Control		77·0	78·6	18·2	15·3	4·8	6·2
Catron	8	3·7	4·1	80·0	82·0	13·0	14·1
Cal. carbimide	50	66·5	65·0	16·4	17·7	8·2†	8·6†
Amphetamine	1	78·4	73·5	16·6	21·0	4·9	5·5
Mescaline	50	76·0	80·0	20·8	16·5	3·4	3·3
Imipramine	20	81·4	78·0	16·4	19·1	2·3	3·1
Tolbutamide	200	73·2	74·6	19·9	19·9	7·3	5·3

* A and B refer to analyses made on sections of chromatograms developed in solvents A and B; see Methods.

† The glucuronide fraction averaged 16·7 per cent of the urinary ^{14}C of which 53 per cent was contributed by NAMT glucuronide. MHPE and an unknown metabolite as their glucuronides were also present. See text.

In order to test the hypothesis that NAMT arises from the demethylation of N-acetylDMPEA, rats were injected with 4 mg and 8 mg N-acetyl-α-^{14}C-DMPEA/kg. Urine was collected for 24 hr and a portion was chromatographed. Only NAMT glucuronide was excreted. It was also observed that within 5 min after the drug was administered (8 mg/kg) the rats became drowsy. Other rats treated with larger amounts (25 and 50 mg/kg) developed marked hypokinesis but remained conscious.

DISCUSSION

Mescaline is converted to its corresponding acid[17] in rats to about the same extent as has been found for DMPEA in this study. In man, approximately 30 per cent of

mescaline[13, 18] and almost all of administered DMPEA[13] is so converted. N-Acetylation provides another pathway for phenethylamine metabolism. While it represents a minor route for catecholamine inactivation, it may have more important implications in polymethoxyphenethylamine metabolism, because N-acetylated products are subsequently O-demethylated. Thus we have found that administered N-acetylDMPEA is excreted only as the conjugated 4-O-demethylated derivative. Similar results have been reported for N-acetylmescaline in the rat[19] and in man.[20] Daly *et al.*[21] have shown that the microsomal and supernatant fraction of rabbit liver converts a small percentage of mescaline to O-demethylated amines. Since microsomal enzymes are surrounded by a lipid barrier, less polar (uncharged) substances should be more easily transported to these enzymes.[22] From the fact that neither 3-methoxytyramine nor homovanillic acid was produced after the administration of DMPEA, whereas NAMT was detected in significant amounts, it can be concluded that N-acetylation precedes O-demethylation. The extensive demethylation of N-acetylDMPEA supports this conclusion. Furthermore, the formation of MHPE (presumably from DMPE), but not methoxytyramine, indicates that only neutral compounds are easily O-demethylated. Similarly, Watabe *et al.*[23] found that the supernatant fraction of rabbit

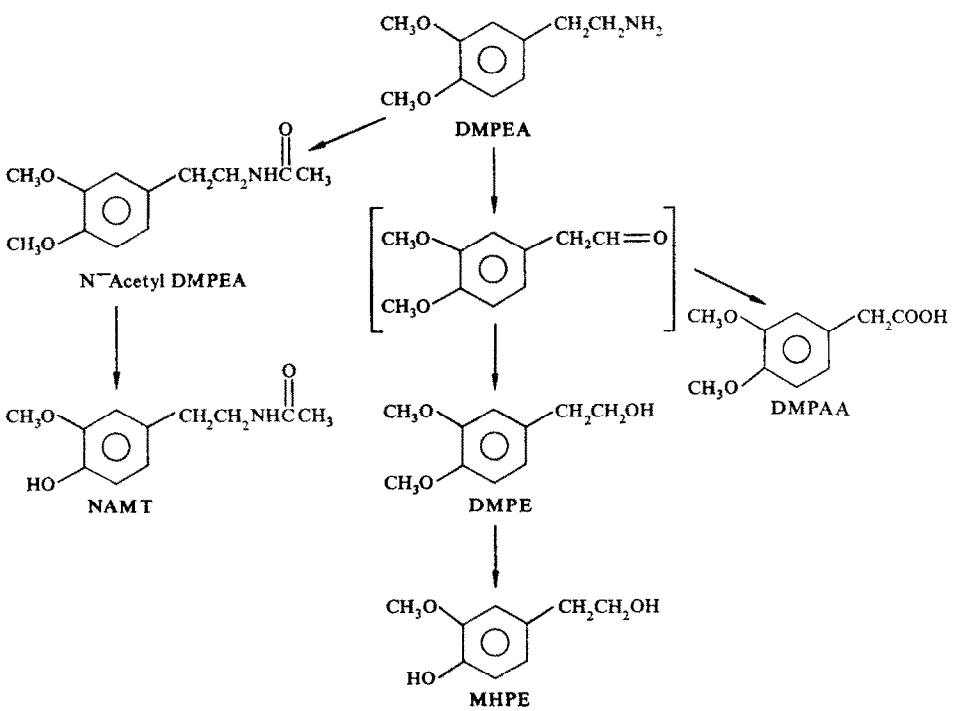

FIG. 1. Metabolic pathways of dimethoxyphenethylamine.

liver largely O-demethylated 3,4,-dimethoxynitrobenzene and that, although N-acetyl-3,4,-dimethoxyaniline underwent deacetylation, in keeping with our observations, the only phenolic metabolite that they isolated also retained the N-acetyl group. The

various metabolites for the transformation of DMPEA are shown in Fig. 1. The metabolites shown in this figure account for 98·5 per cent of the excreted ^{14}C.

The finding that N-acetylDMPEA is physiologically active and that this compound is a metabolic product of DMPEA suggests that this metabolite may be significant in the pharmacological action of DMPEA. It is of interest that N-acetylmescaline appears to be inactive.[20]

Acknowledgement—This study was supported by a Public Health Service Career Development award MH 14024 to Dr. Friedhoff and by Public Health Service Grants MH 07961 and MH 08618.

REFERENCES

1. A. J. Friedhoff and E. Van Winkle, *Nature, Lond.* **194**, 867 (1962).
2. A. J. Friedhoff and E. Van Winkle, *J. nerv. ment. Dis.* **135**, 550 (1962).
3. M. Takesada, Y. Kakimoto, I. Sano and Z. Kaneko, *Nature, Lond.* **199**, 203 (1963).
4. N. P. Sen and P. L. McGeer, *Biochem. biophys. Res. Commun.* **14**, 227 (1964).
5. F. A. Kuehl, Jr., M. Hichens, R. E. Ormond, M. A. P. Meisinger, P. H. Gale, V. J. Cirillo and N. G. Brink, *Nature, Lond.* **203**, 154 (1964).
6. R. E. Bourdillon, C. A. Clarke, A. Ridges, P. M. Sheppard, P. Harper and S. A. Leslie, *Nature, Lond.* **208**, 453 (1965).
7. A. J. Friedhoff and K. Furiya. Submitted for publication.
8. A. Barbeau, J.-A. DeGroot, J.-G. Joly, D. Raymond-Tremblay and J. Donaldson, *Rev. Can. Biol.* **22**, 469 (1963).
9. T. Nishimura and L. R. Gjessing, *Nature, Lond.* **206**, 963 (1965).
10. T. L. Perry, S. Hansen and L. MacIntyre, *Nature, Lond.* **202**, 519 (1964).
11. A. Faurbye and K. Pind, *Acta psychiat. scand.* **40**, 240 (1964).
12. W. V. Studnitz and G. E. Nyman, *Acta. psychiat. scand.* **41**, 117 (1965).
13. A. J. Friedhoff and L. E. Hollister, *Biochem. Pharmac.* **15**, 269 (1966).
14. J. W. Schweitzer and A. J. Friedhoff, *Biochem. biophys. Acta* **111**, 326 (1965).
15. T. Kametani and Y. Nomura, *Yakugaku Kenkyu* **33**, 87 (1961) (*Chem. Abstr.* **55**, 19924).
16. A. A. Smith, M. Fabrykant, M. Kaplan and J. Gavitt, *Biochim. biophys. Acta* **86**, 429 (1964).
17. W. Block, K. Block and B. Patzig, *Hoppe-Seylers Z. physiol. Chem.* **290**, 160 (1952).
18. K. D. Charalampous, A. Orengo, K. E. Walker and J. Kinross-Wright, *J. Pharmac. exp. Ther.* **145**, 242 (1964).
19. J. Musacchio, A. Dudowitz, H. Gerber and M. Goldstein, *Proc. Soc. exp. Biol. Med.* **22**, 481 (1963).
20. K. D. Charalampous, K. E. Walker and J. Kinross-Wright, *Psychopharmacologia, Berl.* **9**, 48 (1966).
21. J. Daly, J. Axelrod and B. Witkop, *Ann. N.Y. Acad. Sci.* **96**, 37 (1962).
22. L. E. Gaudette and B. B. Brodie, *Biochem. Pharmac.* **2**, 89 (1959).
23. T. Watabe, H. Yoshimura and H. Tsukamoto, *Chem. Pharm. Bull.* **12**, 1151 (1964).