A COMPARATIVE STUDY OF MESCALINE AND 3,4-DIMETHOXYPHENYLETHYLAMINE IN ISOLATED BRAIN MITOCHONDRIA AND BRAIN HOMOGENATE

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INTRODUCTION

The role of the brain in the oxidative deamination of polymethoxyphenylethylamines such as mescaline remains unclear. Zeller *et al.*[21] found no evidence of the deamination of mescaline by rabbit brain homogenate although lung and liver homogenates were active. Neff *et al.*[9] reported the presence of 3,4,5-trimethoxyphenylacetic acid (TMPAA), a deaminated product of mescaline, in the brain of the cat after the i.v. injection of labelled mescaline. In the brain, monoamine oxidase resides entirely in mitochondria[11,19]. The experiments reported here were designed to investigate the ability of brain mitochondria to accumulate and to deaminate mescaline and 3,4-dimethoxyphenylethylamine (DMPEA) by employing radiolabelled compounds and a sophisticated analytical technique for the separation of metabolites from unchanged amines. Compounds that influence the active transport mechanisms were used to determine the mode of accumulation of the amines.

MATERIALS AND METHODS

Reagents: [8-^{14}C]mescaline hydrochloride (spec. act. 2.72 mCi/mmole) and [1-^{14}C]DMPEA hydrochloride (spec. act. 9.60 mCi/mmole) were purchased from New England Nuclear Corporation; semicarbazide hydrochloride from Nutritional Biochemical Corporation; ouabain and 2,4-dinitrophenol from Mann Research Laboratories Inc. Iproniazid phosphate (Marsilid®) and reserpine phosphate were generously given by F. Hoffmann–La Roche Inc. and Ciba Pharmaceutical Co., respectively.

Male albino rats and mice weighing from 175 to 200 g and from 30 to 35 g, respectively, were decapitated and the brains immediately removed and homogenized (10%, w/v) in chilled 0.25 *M* sucrose solution. The mitochondria were isolated in a

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Spinco Model L preparative centrifuge (Rotor No. 40) by the method of Løvtrup and Zelander[8]. The separated mitochondria were washed twice, and the particles were suspended in 0.25 *M* sucrose solution (pH 7.5) to give 200 mg of brain tissue per ml of mitochondrial suspension. All procedures were performed at 2°C.

Erlenmeyer flasks (25 ml) containing 1.0 ml of washed mitochondria in 0.25 *M* sucrose solution, 1.0 ml of Tris buffer (0.1 *M*) (pH 7.5), 0.2 ml of sodium chloride solution (15.5%) and 0.5 ml of sucrose solution were incubated for 40 min at 37°C in a Dubnoff metabolic shaker in air. For the drug studies iproniazid (10^{-3} *M*), reserpine (10^{-4} *M*), ouabain (10^{-4} *M*) or 2,4-dinitrophenol (10^{-4} *M*), contained in 0.5 ml of a sucrose solution, was added to the incubation mixture. After an initial 10 min incubation 0.3 ml of a solution containing labelled mescaline (0.3 μCi/23.5 μg) or of labelled DMPEA (0.3 μCi/5.7 μg; 0.9 μCi/17.1 μg; 1.2 μCi/22.8 μg) was added to the flasks and incubation was continued for an additional 30 min. The contents of the flasks were centrifuged (20,000 $\times$ *g*) for 20 min. The supernatants were removed by decantation and saved. The mitochondrial particles were washed with 0.25 *M* sucrose solution and centrifuged to remove labelled amines and metabolites that were either loosely adsorbed to the surface of the mitochondrial particles or trapped by the particles during sedimentation. The levels of total radioactivity in mitochondria before sucrose washes and after 3 successive sucrose washes are shown in Table I. The amounts of radioactivity remaining in mitochondria after the 2nd and 3rd sucrose washes did not differ significantly; as no further significant loss of radioactivity took place, the mitochondrial pellets in subsequent experiments were washed twice with 0.25 *M* sucrose solution.

The radioactivity in the washed mitochondria was extracted with 0.4 *N* perchlor-

TABLE I

THE LEVELS OF TOTAL RADIOACTIVITY IN MOUSE BRAIN MITOCHONDRIA BEFORE AND AFTER SUCROSE WASHES

An incubation medium contained 5.0 ml of Tris–HCl buffer (0.1 *M*) (pH 7.5), 1.0 ml of sodium chloride solution (15.5%), 2.5 ml of sucrose solution and 5.0 ml of mitochondrial suspension (equivalent to 1.0 g of brain tissue). After the initial incubation of 10 min, 1.5 ml of solution containing 1.5 μCi of labelled mescaline or of labelled DMPEA was added to flasks and incubation was continued for an additional 30 min. Aliquots of 3 ml were used for sucrose washes.

	Total radioactivity as disint./min in mitochondria equivalent to 200 mg of wet weight tissue			
	Before sucrose wash	*After first sucrose wash*	*After second sucrose wash*	*After third sucrose wash*
[^{14}C]Mescaline				
Expt. 1	691	658	647	666
Expt. 2	740	705	659	647
[^{14}C]DMPEA				
Expt. 1	3612	3204	3113	3085
Expt. 2	3798	3437	3261	3226

ic acid. The extract was adjusted to pH 5.8 with 5 *N* potassium carbonate solution, and the precipitate of potassium perchlorate was removed by centrifugation. The separation of amines, unchanged mescaline and DMPEA, from their acidic metabolites TMPAA and DMPEAA, in mitochondrial extracts or in incubating media was performed according to the procedure outlined by Charalampous *et al.*[2] using columns 50 mm long with an inside diameter of 4.2–4.5 mm and containing Dowex 50W-X4 (200–400 mesh) previously washed with phosphate buffer (pH 5.8). The evidence for the specificity of the method employed for the separation of amines from acid metabolites is presented elsewhere[13,14]. All extracts were dried in a vacuum desiccator over sulphuric acid at 25°C. The dry residues were re-extracted in 0.5 ml of 50% ethanol and aliquots (250 μl) were assayed for radioactivity in a Nuclear Chicago liquid scintillation spectrometer Model 725 using a method similar to that described previously[15]. All samples were counted for a period of 20 min and in some cases for 40 min to give a maximum of 2% counting error. The efficiency of the counting system ranged between 73 and 78%. The overall recovery of radioactivity was between 80 and 86%. The method of determination of mitochondrial to medium concentration ratios assumes that mitochondria contain 80% water.

RESULTS

Preliminary experiments revealed no significant differences in the levels of DMPEA in mitochondria incubated in the presence of 3 different concentrations (0.3 μCi; 0.9 μCi; 1.2 μCi); the results in Table II are, therefore, reported for mito-

TABLE II

RATIOS OF DMPEAA TO DMPEA IN ISOLATED BRAIN MITOCHONDRIA

The values are reported as ng of DMPEA and DMPEAA in brain mitochondria equivalent to 1 g of wet weight tissue. Each value is an average of 4 separate experiments run in duplicate; values in parentheses represent the ranges.

	Rat			*Mouse*		
	DMPEAA	*DMPEA*	*Ratio DMPEAA: DMPEA*	*DMPEAA*	*DMPEA*	*Ratio DMPEAA: DMPEA*
Control	55.7 (49.8–63)	113.7 (96–126)	32.8 : 67.2	32.0 (26.7–39.9)	91.1 (82.5–105)	26.0 : 74.0
Iproniazid (10^{-3} *M*)	6.4 (5.1– 7.9)	122.5 (110–139)	5.0 : 95.0	12.2 (8.3–14.5)	132.8 (124–141)	9.0 : 91.0
Reserpine (10^{-4} *M*)	63.0 (58 –70)	105.1 (94–108)	37.1 : 69.9	49.6 (45.7–54.9)	87.5 (69.5–94)	36.2 : 63.8
Ouabain (10^{-4} *M*)	46.2 (42.7–56.6)	111.0 (103–125)	29.3 : 70.7	45.6 (37.3–50)	121.0 (105–127)	27.4 : 72.6
2,4-Dinitrophenol (10^{-4} *M*)	53.7 (46.9–58.7)	104.7 (91–121)	33.9 : 66.1	37.9 (31.8–39.7)	91.4 (80–101)	29.3 : 70.7

TABLE III

RATIOS OF TMPAA TO MESCALINE IN ISOLATED MITOCHONDRIA

The values are reported as ng of mescaline and TMPAA in brain mitochondria equivalent to 1 g of wet weight tissue. Each value is an average of 4 separate experiments run in duplicate; values in parentheses represent the ranges.

	Rat			*Mouse*		
	TMPAA	*Mescaline*	*Ratio TMPAA: mescaline*	*TMPAA*	*Mescaline*	*Ratio TMPAA: mescaline*
Control	14.3 (11.5–15.3)	113.0 (102–120)	11.2 : 88.8	8.3 (6.3–11.4)	108.2 (99–112)	7.1 : 92.9
Iproniazid (10^{-3} *M*)	10.0 (8.2–11.7)	128.8 (123–141)	7.2 : 92.8	8.6 (7.1–10.9)	138.3 (132–142)	5.9 : 94.1
Reserpine (10^{-4} *M*)	7.3 (5.9–11.0)	90.6 (83–108)	7.4 : 92.6	10.7 (7.9–11.8)	101.7 (93–108)	9.5 : 90.5
Ouabain (10^{-4} *M*)	11.6 (8.5–13.9)	120.5 (103–127)	8.7 : 91.3	9.1 (8.1–12.3)	93.1 (85–103)	8.9 : 91.1
2,4-Dinitrophenol (10^{-4} *M*)	14.2 (11.8–16.3)	139.6 (123–145)	9.2 : 90.8	8.2 (6.4–13.1)	123.6 (115–127)	6.2 : 93.8

chondria incubated in the presence of 0.3 μCi. The levels of DMPEA and mescaline and of the deaminated metabolites, DMPEAA and TMPAA, in rat and mouse brain mitochondria incubated at 37°C for 30 min in a medium containing [^{14}C]DMPEA or [^{14}C]mescaline are shown in Tables II and III. In rat brain mitochondria the amount of DMPEA was 113.7 ng/g (Table II) and of mescaline 113.0 ng/g (Table III) of wet weight tissue. In mouse brain mitochondria the level of DMPEA, 91.1 ng/g (Table II), was somewhat lower than that of mescaline, 108.2 ng/g (Table III). The concentration of DMPEAA was 55.7 ng/g in rat brain mitochondria and 32.0 ng/g in mouse brain mitochondria (Table II). The amount of TMPAA in rat brain mitochondria was 14.3 ng/g and 8.3 ng/g in mouse brain mitochondria (Table III). The relative concentrations of DMPEAA : DMPEA and TMPAA : mescaline in rat brain mitochondria were 33 : 67 (Table II) and 11 : 89 (Table III) respectively, and 26 : 74 (Table II) and 7 : 93 (Table III) respectively in mouse brain mitochondria. The mitochondrial to medium ratios were determined for both the amines and their acid metabolites. The concentration ratios for nondeaminated drugs were much less than one, ranging between 0.054 and 0.083 for DMPEA and 0.053 and 0.064 for mescaline. The concentration ratios for the acid metabolites were, however, close to unity, ranging between 0.84 and 0.96 for DMPEAA and 1.03 and 1.11 for TMPAA. Iproniazid added in a final concentration of 10^{-3} *M* markedly depressed the formation of DMPEAA in both rat and mouse brain mitochondria (Table II) and lowered the levels of TMPAA in rat brain mitochondria somewhat, although it did not appreciably change the levels in mouse brain mitochondria (Table III). Reserpine in a concentration of 10^{-4} *M* produced no effect on mitochondrial levels of either mescaline or

DMPEA. However, the formation of DMPEAA from DMPEA was somewhat increased. The formation of TMPAA from mescaline was not markedly affected by reserpine. The presence of ouabain (10^{-4} *M*) or 2,4-dinitrophenol in the incubation medium had no noticeable effect on the accumulation of either mescaline or DMPEA although there were slight increases or decreases in the levels of these amines. The omission of Na^+ from the medium or the substitution of K^+, Ca^{2+} or Mg^{2+} for Na^+ had no significant effect on the levels of these psychotomimetic amines in the mitochondria (unpublished data).

In a separate experiment, mouse brain homogenate was incubated at 37°C for 30 min with either mescaline (0.3 μCi) or DMPEA (1.2 μCi) and then subjected to differential centrifugation; the levels of mescaline or DMPEA in mitochondria were almost the same as those reported in Tables II and III.

Mouse brain mitochondria were homogenized in water and then incubated with either [^{14}C]DMPEA or [^{14}C]mescaline. The findings of these experiments are reported in Table IV. The relative concentrations of DMPEAA : DMPEA (28.5 : 71.5), and TMPAA : mescaline (5.5 : 94.5), revealed that on a percentage basis DMPEA and mescaline were metabolized by disrupted mitochondria to the same degree as by intact mitochondria (Tables II and III). Iproniazid (10^{-3} *M*) profoundly depressed the deamination of DMPEA, but had only a slight effect on the deamination of mescaline (Table IV). The formation of DMPEAA and TMPAA in mouse brain homogenate incubated with [^{14}C]DMPEA or with [^{14}C]mescaline for periods of 30, 60 and 120 min are shown in Fig. 1; more DMPEA than mescaline was metabolized by oxidative deamination. Mouse brain homogenate had a limited ability to deaminate mescaline as evidenced by the small amount of TMPAA (5–6%) formed during incubation. The presence of iproniazid (10^{-3} *M*) in the incubation medium markedly lowered the formation of DMPEAA, but did not significantly affect the levels of TMPAA. On the other hand, the presence of semicarbazide (10^{-3} *M*) in the incubation medium had no effect on the metabolism of either DMPEA or mescaline.

TABLE IV

RELATIVE CONCENTRATIONS OF DMPEAA TO DMPEA AND TMPAA TO MESCALINE IN DISRUPTED MITOCHONDRIAL PREPARATION

The mitochondria isolated from mouse brain homogenate were homogenized in distilled water to deliver a mitochondrial suspension equivalent to 200 mg of wet weight brain tissue. The disrupted mitochondria were preincubated at 37°C for 10 min in 0.1 *M* phosphate buffer (pH 7.5) with or without iproniazid followed by [^{14}C]DMPEA (24,360 disint./min) or [^{14}C]mescaline (22,000 disint./min) for 60 min. The reaction was terminated by 0.4 *N* perchloric acid. Each value is an average of 4 separate determinations run in duplicate.

Experiment	*Ratio DMPEAA : DMPEA*	*Ratio TMPAA : mescaline*
Control	28.5 : 71.5	5.5 : 94.5
Iproniazid (10^{-3} *M*)	1.2 : 98.8	4.1 : 95.9

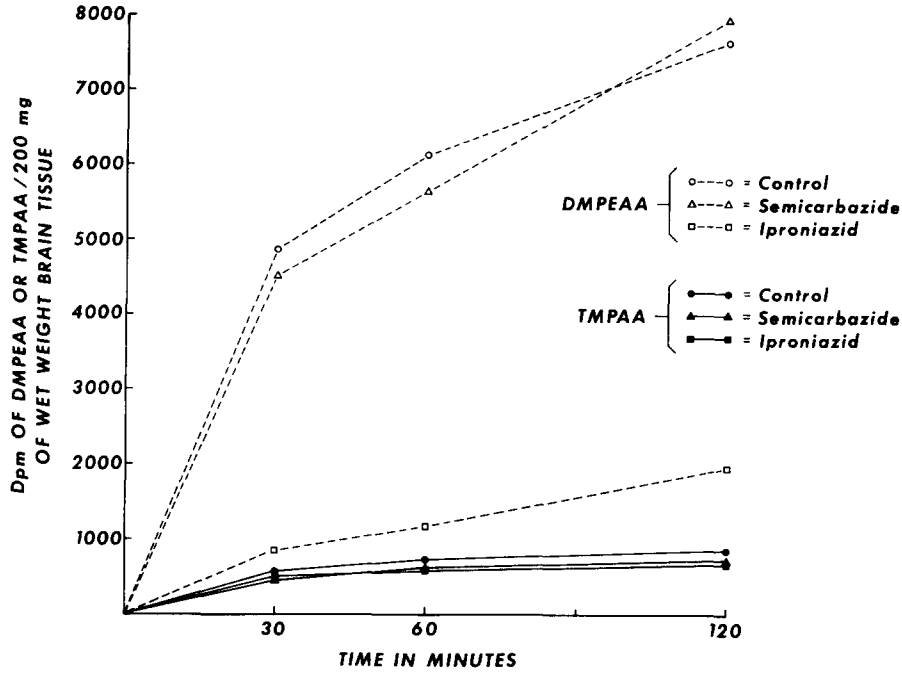

Fig. 1. The sucrose brain homogenate (10% w/v) was centrifuged at 2100 × *g* for 10 min. The supernatant equivalent to 200 mg of brain tissue (wet weight) was preincubated at 37°C for 10 min in 0.1 *M* phosphate buffer (pH 7.5) with or without iproniazid or semicarbazide (10^{-3} *M* in final concentration) followed by [^{14}C]DMPEA (24,360 disint./min) or [^{14}C]mescaline (22,000 disint./min), and the incubation was continued for various periods. The reaction was terminated by 0.4 *N* perchloric acid. Each value on the ordinate is the disint./min recovered in acid metabolites, DMPEAA or TMPAA.

DISCUSSION

A previous report by Vogel[18] showed that brain mitochondria can accumulate a very small fraction of the DMPEA found in the brain after i.p. administration; similar studies *in vivo* but with mescaline have been reported by one of us[12]. The present findings indicate that rat and mouse brain mitochondria incubated at 37°C for 30 min accumulated small amounts of mescaline and DMPEA. However, it is interesting to note that neither the level of DMPEA nor of mescaline in mitochondria was affected by the removal of Na^+ ion from the medium or by the addition of K^+, Ca^{2+} and Mg^{2+} ions to it, indicating that the accumulation of polymethoxyphenylethylamines is independent of the cations. This observation is further confirmed by the addition of ouabain or of dinitrophenol to the incubating media. Ouabain, a potent inhibitor of pump ATPase and an inhibitor of the alkali metal pump on cell membranes[3], and dinitrophenol, which diminishes respiratory ATP formation[10], did not interfere with the accumulation of either mescaline or DMPEA. This could mean that brain mitochondria can accumulate limited amounts of mescaline and DMPEA by a process of simple binding rather than by an active or passive transport mechanism.

Reserpine increased the formation of DMPEAA from DMPEA in both rat and

mouse brain mitochondria, but not that of TMPAA from mescaline. Several studies on the reserpine-treated rat heart[4,6,7] have demonstrated that the initial uptake of norepinephrine across the neuronal membrane of sympathetic nerves is unaffected by reserpine treatment. However, the norepinephrine that enters the sympathetic neurones is rapidly metabolized by monoamine oxidase[5,6] probably because it cannot be protected by binding in the nerve storage granules. In the present investigation, an increased formation of DMPEAA from DMPEA but not of TMPAA from mescaline by brain mitochondria in the presence of reserpine suggests that reserpine may interfere with the binding of DMPEA inside the mitochondrial membrane in a manner similar to that reported for norepinephrine in nerve storage granules. Further experiments are required to validate this assumption.

A comparison of the metabolism of mescaline and of DMPEA by isolated brain mitochondria or whole brain homogenate presents an interesting problem. Considerably more DMPEA than mescaline was deaminated by monoamine oxidase (Table IV, Fig. 1). The presence of iproniazid in the incubating medium effectively depressed the deamination of DMPEA but not that of mescaline. Thus, it might be inferred that DMPEA is a better substrate for brain monoamine oxidase than is mescaline. Previous work by Alles and Heegaard[1] and by Steensholt[16] has shown that with the introduction of an increasing number of methoxy residues on the phenylethylamine molecule, as in polymethoxyamines, the substrate is diverted from monoamine oxidase to a typical diamine oxidase. The work of Zeller[20] has demonstrated that the 3 methoxy residues of mescaline interfere with the interaction between this amine and monoamine oxidase. Recently, Takeo and Himwich[17] reported that a considerably larger dose of DMPEA than of mescaline is required to produce electroencephalographic arousal in the rabbit. They suggest that in the brain either DMPEA is more rapidly inactivated by monoamine oxidase or that it does not easily penetrate the blood–brain barrier. The present findings indicate that, in the brain, monoamine oxidase inactivates DMPEA more rapidly than it does mescaline. Further studies concerned with the relative penetration of psychotomimetic substances and their metabolism in the brain *in vivo* are currently being made.

SUMMARY

The experiments reported here were designed to investigate the ability of isolated brain mitochondria to accumulate labelled mescaline and dimethoxyphenylethylamine (DMPEA). The average concentrations of mescaline and DMPEA accumulated in 30 min at 37°C were 113.0 and 113.7 ng/g in rat brain mitochondria and 108.2 and 91.1 ng/g in mouse brain mitochondria. The concentrations of acid metabolites, trimethoxyphenylacetic acid (TMPAA) and dimethoxyphenylethylacetic acid (DMPEAA), were 14.3 and 55.7 ng/g in rat brain mitochondria and 8.3 and 32.0 ng/g in mouse brain mitochondria. Iproniazid markedly depressed the oxidative deamination of DMPEA but not that of mescaline. Monovalent and divalent cations, ouabain and dinitrophenol exerted no influence on the accumulation of these amines; likewise reserpine was without effect but it somewhat enhanced the formation of DMPEAA.

These findings suggest that brain mitochondria can accumulate these psychotomimetic amines by a process of simple binding rather than by any active transport mechanism.

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