Biochemical Pharmacology, Vol. 23, pp. 273–287. Pergamon Press, 1974. Printed in Great Britain.

OXIDATIVE METABOLISM OF MESCALINE IN THE CENTRAL NERVOUS SYSTEM—IV

IN VIVO METABOLISM OF MESCALINE AND 2,3,4-TRIMETHOXY-β-PHENYLETHYLAMINE*

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(*Received* 29 *March* 1973; *accepted* 25 *June* 1973)

Abstract—Metabolic rates of mescaline (3,4,5-trimethoxy-β-phenylethylamine) and of its non-hallucinogenic isomer, 2,3,4-trimethoxy-β-phenylethylamine were studied in whole and in different areas of mouse brain *in vivo*. The effects of Pargyline and of Probenecid on the concentrations of the amines and their corresponding metabolites, together with the results obtained from intraventricular injections of mescaline suggested the formation of the trimethoxyphenylacetic acids in the brain.

The metabolic differences between mescaline and 2,3,4-trimethoxy-β-phenylethylamine are discussed in terms of possible implications of metabolic parameters with psychotomimetic activity.

D-LYSERGIC acid diethylamide, certain *N,N*-dimethyltryptamines and mescaline have similar central nervous system activity. They produce hallucinations in appropriate doses,[4] and show cross tolerance.[5,6] This suggests a common mechanism for their action. These substances, despite their structural dissimilarities may have common electronic and stereochemical characteristics, which permit them to act on the same biological receptors. However, compounds closely related structurally such as the different trimethoxy-β-phenylethylamines provoke different behavioural changes in experimental animals.[7] In humans mescaline is strongly hallucinogenic,[8] its isomer, 2,3,4-trimethoxy-β-phenylethylamine, however, is not.[9] The electronic structures of these two compounds, among others, have been calculated by the Hückel molecular orbital method, that considers the π-electrons only.[10] Since the side chains of the two compounds are completely identical, this is a permissible simplification. The results obtained by Snyder and Merril suggest that hallucinogenic activity is associated with the high energy of the highest molecular orbital. In other words, the best electron

* Parts of this work were reported at the 16th Psychiatric Research Meeting at Saskatoon, April 1971, and the 3rd International Meeting of the International Society for Neurochemistry, at Budapest July 1971 (see Refs. 2 and 3).

Abbreviations: LSD-25 = D-lysergic acid diethylamide; DANS-Cl = 1-dimethylamino-naphthalene-5-sulphonyl chloride; [^{3}H]3,4,5-TMPEA = 2,6[^{3}H]3,4,5-trimethoxy-β-phenylethylamine = [^{3}H]mescaline; [^{14}C]3,4,5-TMPEA = 8[^{14}C]mescaline; [^{3}H]2,3,4-TMPEA = 5,6[^{3}H]2,3,4-trimethoxy-β-phenylethylamine; [^{3}H]3,4,5-TMPAA = 2,6[^{3}H]3,4,5-trimethoxy-phenylacetic acid; [^{14}C]3,4,5-TMPAA = 8[^{14}C]3,4,5-trimethoxy-phenylacetic acid; PPO = 2,5-diphenyloxazol; POPOP = 1,4-bis-[2-(5-phenyloxazolyl)]-benzene; MAO = monoamine oxidase; DAO = diamine oxidase; CSF = cerebrospinal fluid; TLC = thin-layer chromatography; TLE = thin-layer electrophoresis; MS = mass spectrometry; S.E.M. = standard error of the mean value;

donor and the poorest electron acceptor are the most potent hallucinogens. Such relationships seem to hold also for methoxy-amphetamines, as total valence electron calculations by Kang and Green[11] have demonstrated.

Although there is no reason to doubt that electron donor–acceptor properties of a given molecule are decisive for specific interactions with receptors, hallucinogenic properties may be perhaps more actively dictated by other factors, for instance permeation of the blood–brain barrier, permeation of nerve cell membranes, or distribution and metabolism in brain and other organs. It is not certain at present, whether mescaline itself or a mescaline derivative is the active hallucinogenic compound. Block and coworkers,[12] (more than 20 years ago) suggested that protein bound mescaline is the active principle, but Friedhoff and Goldstein,[13] believe that the aldehyde formed by oxidative deamination of mescaline or the alcohol obtained by reduction of 3,4,5-trimethoxyphenylacetaldehyde are related to the psychotomimetic effect of mescaline. Even if we assume from certain observations[14,15] that mescaline itself reacts directly with certain receptors, thus causing the psychotomimetic effects, kinetic parameters of mescaline metabolism may nevertheless have decisive influence on its pharmacologic activity. Data on the metabolism of mescaline and its isomers, and also of the hallucinogenic amphetamines, however is lacking, especially for brain,[8,16,17] so that there is ample room for speculation.[14,18]

Recent investigations to clarify and understand the mode of action of psychotomimetic compounds were based on the conception of a direct interaction with certain receptors of these compounds, although, especially in the case of mescaline and the amphetamines, metabolic steps between application and action seem to be probable.[19,20]

The papers of Shah and Himwich[21–23] which partially overlap with our publications on this topic[2,3,24,25] threw some light on the oxidative metabolism of mescaline in rat and mouse brain. Nevertheless, mescaline metabolism in brain is still not completely clear.

Autoradiographs of mouse and monkey brain, obtained in our laboratory[26,27] demonstrated 6 hr after mescaline intake a marked accumulation of radioactivity in the hippocampus and in the putamen region and in smaller amounts also in other cortical areas, the rest of the brain being apparently devoid of radioactivity. Comparable distributions of radioactivity were found by other groups using psilocybin and LSD, and some compounds which prevent mescaline hallucinations, such as chlorpromazine.[28–35] We also observed 5–8 hr after mescaline administration to mice an increased motor activity, following the tranquil period 3–5 hr after the injection.[26] We therefore studied the regional metabolism of mescaline in mouse brain and compared it with its non-hallucinogenic isomer, 2,3,4-trimethoxy-β-phenylethylamine.

MATERIALS AND METHODS

Radiochemicals. 8[^{14}C]mescaline.HCl (specific activity 4·5 mCi/mmole) was purchased from New England Nuclear Corporation, Boston.

2,6[^{3}H]mescaline.HCl (specific activity 54·1 mCi/mmole), ([^{3}H]3,4,5-TMPEA), 5,6[^{3}H]2,3,4-trimethoxy-β-phenylethylamine.HCl (specific activity 15·4 and 75·7 mCi/mmole) ([^{3}H]2,3,4-TMPEA), and 2,6[^{3}H]3,4,5-trimethoxyphenylacetic acid (specific activity 64·2 mCi/mmole) ([^{3}H]3,4,5-TMPAA) were prepared by electrophilic substitution of the corresponding non-radioactive compounds with BF_3 saturated phosphoric acid in tritiated water according to Seiler *et al.*[36] Purification was achieved

by recrystallization from ethyl acetate–ethanol. The specificity of the nuclear tritiation was examined by reaction of the [^{3}H]labelled compounds with Br_2 in water at room temperature. Tritium was substituted by bromine under these conditions by more than 99 per cent. Uniformity of the two β-phenylethylamines was checked by reaction with DANS-Cl, subsequent TLC of the DANS-amides in several solvents, and by MS. To check the eventual tritium exchange from [^{3}H]3,4,5-TMPAA, a mixture of [^{3}H]3,4,5-TMPAA and [^{14}C]3,4,5-TMPAA was prepared by enzymatic oxidation of a mixture of [^{3}H] and [^{14}C]mescaline with rat liver mitochondria in 0·066 M phosphate buffer pH 8·3. The acid mixture was isolated by TLC and TLE.

Inhibitors and reagents. Pargyline® (*N*-methyl-*N*-benzyl-propargylamine.HCl) (Abbott Laboratories, North Chicago), semicarbazide.HCl (E. Merck, Darmstadt) and Probenecid [(*p*-(dipropylsulfamoyl)-benzoic acid] (Sharp and Dohme, GmbH, München) were purchased from the respective producer. The usual laboratory chemicals were from E. Merck, Darmstadt.

Experimental animals. Male albino mice (NMRI, Süddeutsche Versuchstierfärm, Tuttlingen, or Gesellschaft für Versuchstierzucht, Hannover) (30–35 g) were used. The animals were housed in standard cages in groups of about 10; they were fed Altromine standard diet and water *ad lib.* during the whole experiment, if the time between administration of the radioactive compound and decapitation exceeded 1 hr.

Administration of radioactive material and tissue extraction. Radioactive compounds were normally injected intraperitoneally in a dose of 120 mg/kg. The material was dissolved in 0·2 ml distilled water. Intraventricular injections were performed under chloralhydrate (15 mg/animal) narcosis.[37,38] The radioactive compounds were dissolved (25 μg/20 μl) in distilled water.

Pargyline was administered intraperitoneally in two doses each 50 mg/kg, 24 and 12 hr before the administration of the radioactive compounds. 50 mg/kg semicarbazide.HCl were injected intraperitoneally 3 hr, and Probenecid (360 mg/kg) 1 hr before the injection of the radioactive material. Pargyline and semicarbazide.HCl were dissolved in 0·2 ml distilled water, Probenecid was dissolved in 0·2 M K_2CO_3 solution. This solution was brought to pH 7·3–7·5 with KH_2PO_4 solution.

Groups of three animals were killed by decapitation at different time intervals (between 1 and 24 hr) after the administration of the radioactive compounds. Brains (and in one instance several other organs) were removed and frozen immediately with dry ice. Until dissection and homogenization, the brains were stored in sealed plastic bags at −20°. For regional studies the following parts were dissected at −15° with a small scalpel: neocortex, hippocampus, the region around N. amygdalae, thalamus, hypothalamus, and cerebellum. The different preparations were controlled by histological methods and found to be sufficiently pure for the intended experiments.

Whole brains were homogenized with 5 parts of 0·2 N $HClO_4$. The small pieces of the different brain regions (5–50 mg) were repeatedly frozen and thawed in 0·3 ml 0·2 N $HClO_4$, and then disrupted by vigorous stirring with a Whirlmix (Cenco, Breda). The supernatants of the homogenates ("tissue extracts") were subjected to different separation procedures.

Analytical procedures

Separation of tissue extracts into anionic plus neutral and cationic fractions, and determination of radioactivity in these fractions. The method used in the present paper

for the separation of the anionic plus neutral and the cationic mescaline metabolites was essentially the same as that described in detail previously.[1,24] Aliquots (0·3-ml) of neutralized tissue extracts were applied to 4 × 100 mm cellulose phosphate columns. A device was constructed, which allowed the handling of 20 columns at the same time. Suction velocity was individually regulated with needle valves.

Elution of the anionic plus neutral compounds of the tissue extracts was accomplished by successive washings with 1 ml of ethanol and 1 ml of methanol. For the elution of the cationic components 1 ml of a mixture of ethanolamine–methanol (1:1) was used, followed by 2 ml of ethanol.

The column eluates were mixed with 10 ml of scintillator cocktails (50 g naphthalene, 7 g PPO and 50 mg POPOP/l dioxane for the anionic plus neutral compounds; 5 g PPO/l toluene for the cationic fractions). A Packard TriCarb (model 3375) was used for liquid scintillation spectrometry.

Since tritium exchange of the [^{3}H]labelled β-phenylethylamines and the [^{3}H]3,4,5-TMPAA could not be excluded in advance, mixtures of 8[^{14}C]mescaline.HCl and 2,6[^{3}H]mescaline·HCl, and of 8[^{14}C]3,4,5-TMPAA and 2,6[^{3}H]3,4,5-TMPAA were dissolved in 0·3 ml 0·2 N $HClO_4$ and treated in the same manner as the tissue extracts. Determinations of ^{3}H/^{14}C-ratios before and after the processing showed no measurable tritium losses. These experiments showed a recovery of radioactivity of 98 ± 4 per cent, both of the amine and the acid.

Determination of the acid metabolites of mescaline. After neutralization and deproteinization with ethanol tissue extracts were applied in 3 cm long streaks to thin-layer plates (200 μm silica gel G_{F254}, E. Merck, Darmstadt), with an Autoliner (Desaga, Heidelberg). The plates were developed with tert. butanol-2-oxobutane–25% ammonia–water (4:3:2:1). *N*-acetyl-mescaline, the main neutral metabolite of mescaline[1] is not separated clearly from mescaline in this solvent mixture. 10 × 35 mm zones were scraped off along the length of the chromatographic plate. The silica gel of each zone was extracted with 2 × 1 ml methanol–ethanol (1:1). Radioactivity was determined in the extracts after addition of 10 ml PPO in toluene. The percentage of radioactivity present in the acids was calculated from the total radioactivity along the length of the chromatogram. Absolute values were obtained by determination of the yield of radioactivity after chromatographic separation, by comparison with the total radioactivity of the tissue homogenate.

Determination of non-extractable radioactivity. 0·5 ml portions of the tissue homogenates were centrifuged and the precipitates washed with 4 × 1 ml 0·2 N $HClO_4$. After four washings with perchloric acid, no radioactivity was detectable in the supernatant. The acid insoluble tissue residue was suspended in 0·5 ml 1 N NaOH and incubated at 38° until the proteins were solubilized. 0·5 ml of 96% formic acid was added, and 12 hr later the solution was mixed with 10 ml Aquasol® (New England Nuclear Corporation, Boston), or with 10 ml of a solution containing 300 ml Triton X100 and 5 g PPO/l. toluene. Radioactivity was determined in these samples as usual.

RESULTS

Oxidative metabolism of mescaline and 2,3,4-trimethoxy-β-phenylethylamine in whole brain

There are obvious differences in the metabolic rates of the two closely related amines, 3,4,5-TMPEA and 2,3,4-TMPEA in whole brain. Table 1 shows the con-

centrations of mescaline, 2,3,4-TMPEA and of their anionic plus neutral metabolites in whole mouse brain, during the 24 hr period, after a single intraperitoneal injection of [^{3}H]3,4,5-TMPEA and [^{3}H]2,3,4-TMPEA. It is apparent from the data in Table 1 that the metabolites of mescaline were present in brain in low concentrations throughout the 24 hr, whereas a high metabolite concentration was detectable during the first 3 hours after 2,3,4-TMPEA administration. However, 24 hr after the injections, no differences in the concentrations of the metabolites of the two β-phenylethylamines was observed. The amine concentrations behaved inversely. One hr after administration the concentration of mescaline was higher than the concentration of 2,3,4-TMPEA but again the amine concentrations were equally low after 24 hr.

TABLE 1. CONCENTRATION OF 3,4,5-TRIMETHOXY-β-PHENYLETHYLAMINE (MESCALINE), 2,3,4,-TRIMETHOXY-β-PHENYLETHYLAMINE, AND THEIR ANIONIC PLUS NEUTRAL METABOLITES IN BRAIN, AFTER A SINGLE INTRAPERITONEAL INJECTION OF 2,6[^{3}H]MESCALINE.HCl (120 mg/kg; 0·87 mCi) AND 5,6[^{3}H]-2,3,4-TRIMETHOXY-β-PHENYLETHYLAMINE.HCl (120 mg/kg; 0·25 mCi), RESPECTIVELY

	3,4,5,-Trimethoxy-β-phenylethylamine (mescaline)			2,3,4-Trimethoxy-β-phenylethylamine		
Time after inj. (hr)	Cationic fraction (nmole/g)	Anionic + neutral fraction (nmole/g)	Cationic / Anionic + neutral	Cationic fraction (nmole/g)	Anionic + neutral fraction (nmole/g)	Cationic / Anionic + neutral
1	40·7 ± 12·9	3·1 ± 0·7	13·1	24·7 ± 10·4	63·5 ± 7·7	0·39
3	13·2 ± 4·2	2·2 ± 0·3	6·0	4·8 ± 3·5	16·2 ± 4·8	0·30
6	2·4 ± 0·5	2·1 ± 0·2	1·1	3·0 ± 0·2	2·7 ± 0·4	1·1
12	0·7 ± 0·4	1·7 ± 0·4	0·41	0·9 ± 0·3	2·3 ± 0·4	0·39
24	0·5 ± 0·1	1·9 ± 0·3	0·26	0·7 ± 0·2	1·6 ± 0·3	0·44

Mean values of three experiments ± S.E.M.

It should be noted that independent of the amount of mescaline applied, and independent of the mode of application, there is always a constant proportion of this compound in mouse brain. We can calculate from the figures of Table 1 a half life of mescaline in mouse brain of approximately 60 min.

The figures of Table 1 were obtained by ion exchange column chromatography of the perchloric acid brain extracts. Essentially the same results were obtained, as far as mescaline and 2,3,4-TMPEA concentrations are concerned, if the acidic metabolites were separated from the amines by TLC, as can be seen in Table 2. However, there is a significant difference between the results obtained by ion exchange chromatography and by TLC. While the concentration of the acidic mescaline metabolites decreased from 2·9 to 0·2 nmole/g between 1 and 6 hr after mescaline administration (Table 2) the decrease in the anionic plus neutral metabolites measured after ion exchange column separation was much lower (Table 1). The difference in the results obtained with the two methods was, however, not observed in case of the metabolites of 2,3,4-TMPEA. We can derive from these observations, that *N*-acetylation plays a more important role in mescaline metabolism than in the metabolism of 2,3,4-TMPEA.

The influence of a MAO inhibitor (Pargyline) and of a DAO inhibitor (semicarbazide·HCl) on the concentrations in brain of 3,4,5-TMPEA, 2,3,4-TMPEA and

TABLE 2. INFLUENCE OF PARGYLINE® AND SEMICARBAZIDE TREATMENT ON THE CONCENTRATIONS IN MOUSE BRAIN OF MESCALINE (3,4,5-TRIMETHOXY-β-PHENYLETHYLAMINE), 2,3,4-TRIMETHOXY-β-PHENYLETHYLAMINE AND OF THEIR RESPECTIVE ACIDIC METABOLITES

Treatment	Time after inj. (hr)	3,4,5-Trimethoxy-β-phenylethylamine Mescaline			2,3,4-Trimethoxy-β-phenylethylamine		
		Amine (nmole/g)	Acids (nmole/g)	Amine / Acids	Amine (nmole/g)	Acids (nmole/g)	Amine / Acids
Untreated	1	40·4 ± 10·5	2·9 ± 0·6	13·9	35·6 ± 5·1	66·2 ± 32·7	0·59
control	3	11·5 ± 2·6	0·8 ± 0·3	14·4	5·0 ± 1·0	14·1 ± 6·1	0·35
group	6	2·3 ± 0·05	0·2 ± 0·1	11·5	2·6 ± 0·9	3·2 ± 0·6	0·81
Pargyline	1	55·3 ± 7·9	1·5 ± 0·4	36·9	247 ± 54	9·8 ± 1·0	25·1
2 × 50 mg/kg	3	15·8 ± 3·2	0·4 ± 0·1	39·4	104 ± 22	10·0 ± 1·3	10·4
	6	3·6 ± 0·4	0·1 ± 0·02	36·0	7·1 ± 0·9	0·9 ± 0·1	7·9
Semi-carbazide.HCl	1	58·2 ± 0·8	2·0 ± 1·0	29·0	41·6 ± 22	48·5 ± 11	0·85
50 mg/kg	3	23·4 ± 2·9	1·0 ± 0·3	23·4	3·6 ± 0·2	15·7 ± 4·8	0·23
	6	4·6 ± 0·2	0·2 ± 0·1	23·0	2·2 ± 0·5	2·8 ± 0·6	0·79

Mean values of three experiments ± S.E.M.

of their respective acidic metabolites, can also be seen in Table 2. MAO inhibition elevated the mescaline concentration in mouse brain, and decreased the acidic metabolites. These effects are, however, much more pronounced in the case of the mescaline isomer. One hr after the administration of 2,3,4-TMPEA the amine concentration was elevated by approximately 700 per cent as compared with untreated controls. The decrease in amine concentration is also considerably slower in the Pargyline treated animals. Semicarbazide treatment on the other hand had apparently no effect on 2,3,4-TMPEA metabolism. In the case of mescaline, however, amine concentration was increased, and the acids concomitantly decreased, at least during the first hour after the mescaline administration, the effects being approximately the same as after Pargyline treatment. Total radioactivity of blood after [^{3}H]3,4,5-TMPEA and [^{3}H] 2,3,4-TMPEA administration was not altered significantly by Pargyline or semicarbazide treatment (Table 3). However, radioactivity in the acid insoluble brain components was significantly influenced by pretreatment of the animals with MAO and DAO inhibitors, as shown in Fig. 1. In untreated controls the radioactivity of the acid insoluble tissue components was maximal approximately 3 hr after mescaline administration. This was also the case in the semicarbazide treated animals, however, with somewhat increased radioactivity. In Pargyline pretreated animals a steady decline of radioactivity in the acid insoluble material was observed. Based on the total amount of radioactivity present in brain at a certain time, the bound radioactivity increased with time, independent of the pretreatment.

Different relationships were found in the 2,3,4-TMPEA injected animals. Untreated controls and semicarbazide treated animals showed a rapid decline of bound radioactivity with time, whereas in Pargyline treated mice radioactivity in the acid insoluble brain components 3 hr after the administration of the amine was relatively high. On an absolute scale, the amount of acid insoluble radioactivity was at any time larger than in the corresponding experiments with radioactive mescaline. Based on the total

TABLE 3. TOTAL RADIOACTIVITY IN BLOOD AND BRAIN AFTER THE INTRAPERITONEAL ADMINISTRATION OF 120 mg/kg (0·87 mCi) 2,6[^{3}H]MESCALINE·HCl, AND 120 mg/kg (0·25 mCi) 5,6[^{3}H]2,3,4-TRIMETHOXY-β-PHENYLETHYLAMINE·HCl, RESPECTIVELY; INFLUENCE OF PRETREATMENT WITH PARGYLINE AND SEMICARBAZIDE

Treatment	Time after inj. (hr)	3,4,5-Trimethoxy-β-phenylethylamine (mescaline) Brain (dis/min/mg)	Blood (dis/min/mg)	Brain/Blood	2,3,4-Trimethoxy-β-phenylethylamine Brain (dis/min/mg)	Blood (dis/min/mg)	Brain/Blood
Untreated control group	1	5050 ± 140	15,380 ± 4400	0·33	4150 ± 1680	15,580 ± 6740	0·27
	3	1450 ± 340	1270 ± 860	1·1	920 ± 330	7000 ± 3680	0·13
	6	360 ± 100	360 ± 70	1·0	270 ± 20	1860 ± 260	0·14
Pargyline 2 × 50 mg/kg	1	6590 ± 1250	8530 ± 1770	0·77	12,440 ± 2590	13,840 ± 240	0·90
	3	1930 ± 380	1120 ± 300	1·7	5530 ± 1170	6150 ± 2480	0·90
	6	440 ± 50	350 ± 70	1·2	420 ± 20	2200 ± 450	0·19
Semicarbazide.HCl 50 mg/kg	1	7190 ± 500	13,660 ± 5680	0·52	4380 ± 920	26,890 ± 4490	0·16
	3	2950 ± 370	1660 ± 330	1·8	940 ± 240	10,710 ± 180	0·09
	6	580 ± 10	360 ± 150	1·6	240 ± 3	2020 ± 520	0·12

Mean values of three experiments ± S.E.M.

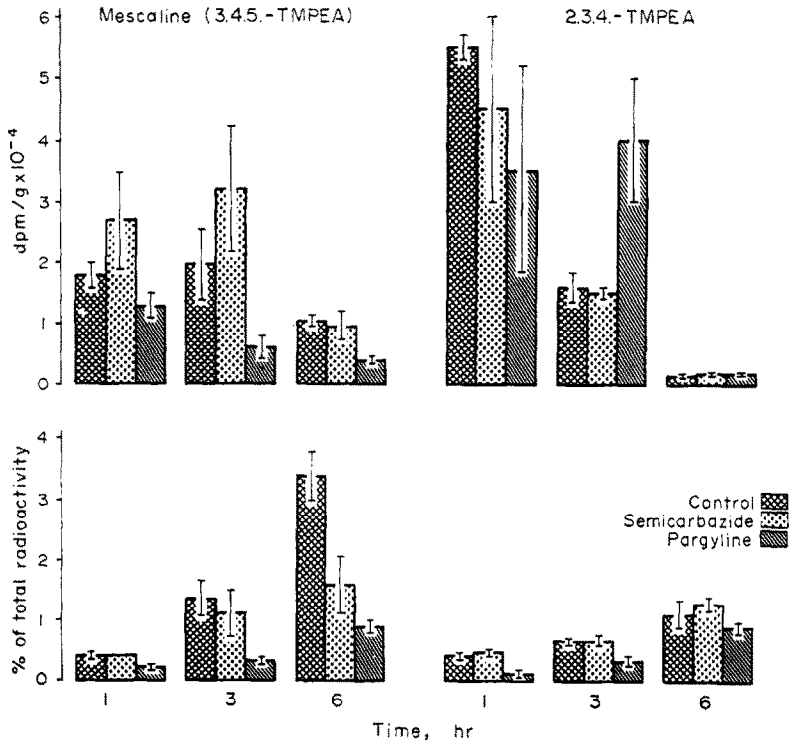

FIG. 1. Radioactivity in the perchloric acid insoluble tissue constituents of brain after the intraperitoneal injection of [^{3}H]mescaline and [^{3}H]2,3,4-TMPEA into untreated controls and to animals pretreated with Pargyline and semicarbazide·HCl. Ordinate: Total amount (dis/min/g brain) and relative amount (per cent of the radioactivity present in brain). Abscissa: Time after the injection of the radioactive compounds.

amount of radioactivity in the brain, the relative amount of bound radioactivity was, however, lower, than in case of mescaline and, there was no increase in the relative amounts of bound radioactivity with time. In this respect the Pargyline treated animals were not exceptional.

The origin of the acidic metabolites of 3,4,5-*TMPEA and* 2,3,4-*TMPEA*

It is still not certain whether the acidic metabolites of mescaline are formed in brain or in peripheral organs.[22] A series of experiments was carried out therefore, in order to clarify this problem.

It can be derived from the data of Table 4 that a small amount of intraperitoneally injected [^{3}H]3,4,5-TMPAA penetrates into the brain, this amount being directly related to the injected amount and the blood concentration of 3,4,5-TMPAA, respectively.

TABLE 4. THE LEVELS OF 2,6[^{3}H]3,4,5-TRIMETHOXY-PHENYLACETIC ACID IN BLOOD AND BRAIN 10 MIN AFTER ITS INTRAPERITONEAL INJECTION

Injected amount of [^{3}H]-3,4,5-TMPAA (nmole)	Blood 3,4,5-TMPAA (nmole/g)	Brain 3,4,5-TMPAA (nmole/g)	Percent of the injected radioactivity (per g brain)	Brain / Blood
400	7·0 ± 1·2	0·19 ± 0·06	0·047	0·027
2000	30·6 ± 8·8	0·93 ± 0·06	0·047	0·031
4000	93·4 ± 27·2	1·61 ± 0·86	0·040	0·017

Mean values of three experiments ± S.E.M.

[^{3}H]Mescaline.HCl was injected intraventricularly in amount (25 μg/animal) comparable to that which can be expected in the brain 10 min after the intraperitoneal injection of 120 mg/kg. The concentration profiles of mescaline and of its metabolites were followed over a period of 6 hr. The results of these experiments are summarized in Table 5. Comparison of the figures of Table 5 with those of Table 2 shows very similar concentration profiles of mescaline and its acidic metabolites in brain, after intraperitoneal and intraventricular mescaline administration, although the brain–blood ratios of total radioactivity differ very considerably after the two modes of mescaline administration, as can be derived from Tables 3 and 5.

TABLE 5. CONCENTRATION OF MESCALINE AND OF ITS ACIDIC METABOLITES IN BRAIN AFTER A SINGLE INTRAVENTRICULAR INJECTION OF 25 μg (5·4 μCi) 2,6[^{3}H]MESCALINE. HCl

Time after injection (hr)	Mescaline (nmole/g)	Acids (nmole/g)	Mescaline / Acids	Brain / Blood (Total radioactivity/mg)
1	46·5 ± 13	2·8 ± 0·2	16·8	2·4
3	9·2 ± 2·8	0·7 ± 0·2	13·1	1·9
6	6·8 ± 2·8	0·5 ± 0·06	13·5	0·5

Mean values of three experiments ± S.E.M.

The effect of pretreatment of the animals with 360 mg/kg Probenecid before intraperitoneal injection of mescaline is shown in Fig. 2. While in the untreated controls the acidic metabolites—rapidly declined between 1 and 3 hr after the mescaline administration, as was shown already in Tables 1 and 2, the concentration of the acidic metabolites increased considerably with time in the Probenecid treated animals. Consequently the ratio of acidic metabolites/mescaline increased at the same time.

It should be mentioned that treatment with high Probenecid doses did not only influence the brain to plasma transport of the acidic metabolites. It also influenced the brain levels of mescaline. One hour after mescaline administration we found only approximately 50 per cent of the mescaline concentration in the brain of animals

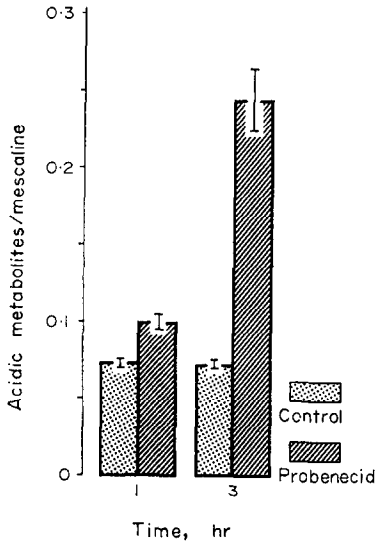

FIG. 2. Effect of pretreatment with Probenecid on the accumulation of acidic mescaline metabolites in mouse brain. Ordinate: concentration ratio of acidic metabolites to mescaline; abscissa: time after the administration of [^{3}H]mescaline.

pretreated with 360 mg/kg Probenecid, as compared with untreated controls. It should be noted furthermore that the Probenecid effect does not last long. Doses of 180 mg/kg were active for approximately 60 min, whereas a dose of 360 mg/kg caused the elevation of the levels of the acidic metabolites for more than 3 hr.

Regional metabolism of mescaline and 2,3,4-trimethoxy-β-phenylethylamine in mouse brain

In Fig. 3 the time course of total radioactivity, of mescaline, and its acidic plus neutral metabolites is demonstrated in some brain regions. Each point of the curves represents the average value of three to six animals, and the bars the maximal deviations measured. The hypothalamus contained initially the highest content of mescaline. There was a relatively small decrease in the mescaline concentration in hippocampus and in the amygdala region during the first 3 hr; a bigger decline in the mescaline content recurred in the other regions. However 6 hr after mescaline injection the amine

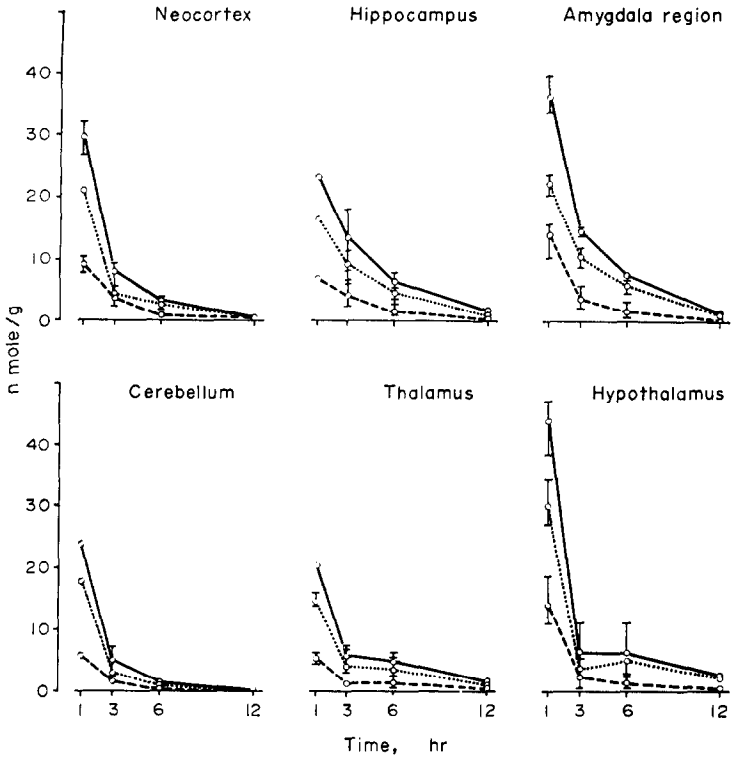

FIG. 3. Time course of the total radioactivity and of the concentrations of mescaline and its acidic metabolites in mouse brain regions following the intraperitoneal injection of [^{3}H]mescaline·HCl.

concentration in the neocortical structures and cerebellum differed considerably from that in the hippocampus, amygdala region, thalamus and hypothalamus concentrations in the latter structures being at least twice as high at this time as those in neocortex and cerebellum. This difference was still observed 12 hr after mescaline administration. As was observed with whole brain, the amine concentrations always exceeded those of the mescaline metabolites.

A completely different relationship was observed, however, following the injection of 2,3,4-TMPEA (Fig. 4). The total amount of radioactivity observed in the selected brain areas was considerably higher than after mescaline administration. This radioactivity, however, was due mainly to metabolites of the amine. The amine content at any time was very low, as compared with mescaline.

DISCUSSION

We have demonstrated previously that beside of the well known mescaline metabolite 3,4,5-TMPAA, 3,4,5-trimethoxy-benzoic acid is formed also from mescaline *in vivo*, both in brain and in visceral organs.[1] The analytical methods currently used cannot distinguish between these acids. In fact sophisticated methods are needed to determine 3,4,5-TMPAA and 3,4,5-trimethoxy-benzoic acid mixtures quantitatively in tissue extracts. In order to indicate that the sum of the two acids has been determined, we use in the present work the terms acidic metabolites or anionic fraction. With the

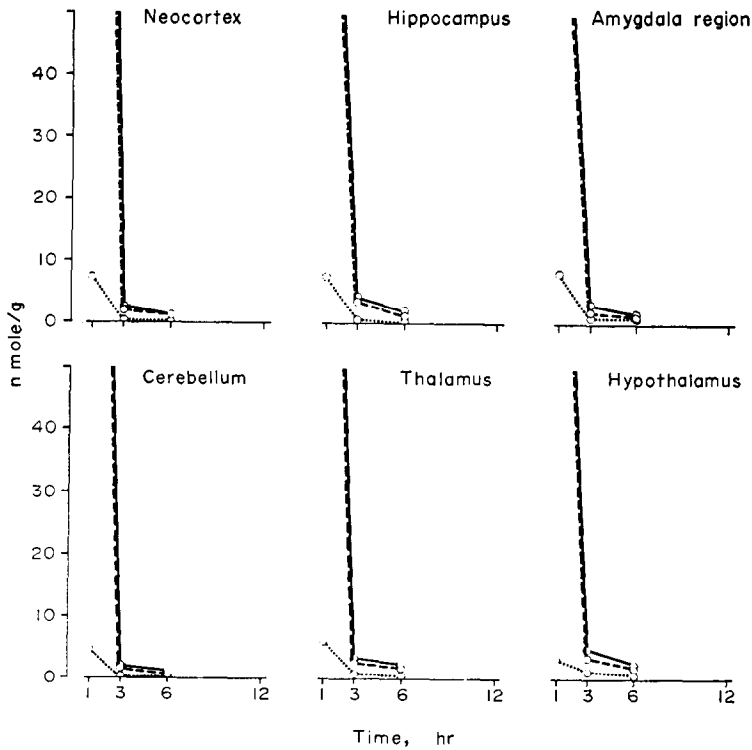

FIG. 4. Time course of the total radioactivity and of the concentrations of 2,3,4-TMPEA and its acidic metabolites in mouse brain regions following the intraperitoneal injection of [^{3}H]2,3,4-TMPEA.

ion exchange column technique even the neutral metabolites of mescaline, mainly *N*-acetyl-mescaline,[1] were determined together with the acids. The acidic plus neutral fraction represents essentially the metabolized portion of mescaline. Cationic metabolites are formed from mescaline only in low amounts.[1]

Our knowledge of the dynamics and enzymology of mescaline metabolism in brain *in vivo* is scarce. We concluded on the basis of the inhibitory spectrum, as observed with brain homogenates[24,25] that mescaline can be oxidized to 3,4,5-TMPAA in brain by an enzyme most probably identical with mitochondrial MAO. The same conclusion was drawn subsequently by Shah and Himwich.[22] The mescaline isomer, 2,3,4-TMPEA, however, was much more rapidly oxidized *in vitro* than mescaline, and in accordance with some other MAO substrates, this compound inhibits its own enzymatic degradation at high substrate levels to a degree, which corresponds approximately to the maximal rate of mescaline oxidation in homogenates.[24] Corresponding differences between the two related amines can be observed *in vivo*: 2,3,4-TMPEA is much more rapidly oxidized than mescaline, as can be derived from the high concentration of acidic metabolites (mainly 2,3,4-TMPAA), and the relatively low amine concentration in brain, following the intraperitoneal injection of this compound (Table 1). From the sharp increase in amine concentration in brain, and the concomitant decrease in the acidic metabolites after Pargyline pretreatment (Table 2) it can be concluded, that 2,3,4-TMPEA is mainly metabolized by mitochondrial MAO.

Compared with 2,3,4-TMPEA, mescaline shows some noticeable differences: (1) The amine concentration is always higher than the concentration of the acidic metabolites (Table 1). The well measurable difference between the experiments in which the neutral plus acidic metabolites were measured (Table 1) and those, in which only the acids were determined (Table 2) suggest a considerable formation of neutral metabolites, mainly *N*-acetyl-mescaline, while this reaction is probably of little significance in case of 2,3,4-TMPEA metabolism. (2) Pargyline pretreatment has a pronounced effect on the amine concentration in brain. Semicarbazide pretreatment also raises the mescaline concentration, without, however, decreasing significantly the concentrations of the acids below the control values (Table 2). *In vitro* semicarbazide and other DAO inhibitors have only slight influence on mescaline oxidation.[24,25] These observations, together with the relatively rapid elimination of mescaline from brain suggest several pathways of mescaline metabolism: oxidation by MAO; oxidative degradation to 3,4,5-trimethoxy-benzoic acid;[1] *N*-acetylation; and probably transamination, besides secernation into the CSF and outflow from brain with the blood stream. Evidence for mescaline transamination is very indirect at present. We assume this type of reaction on grounds of the *in vivo* effect of semicarbazide on mescaline metabolism, the slight inhibitory activity of DAO inhibitors on mescaline degradation by brain homogenates and furthermore on the lack of effect of DAO inhibitors on 2,3,4-TMPEA metabolism *in vitro* and *in vivo*.

There is not much doubt about the formation of 2,3,4-TMPAA in brain after the intraperitoneal administration of 2,3,4-TMPEA; the effect of Pargyline especially provides good evidence. The data of Table 2 indicate that 2,3,4-TMPAA elimination from brain cannot keep pace with its formation.

Despite the Pargyline effect on mescaline metabolism, we are still doubtful about the formation of 3,4,5-TMPAA in brain, and consequently about the correctness of the suggestions concerned with brain metabolism of mescaline, made above. Indeed there was no convincing argument against the assumption that the metabolites of mescaline found in brain were of peripheral origin.[22] It was necessary, therefore, to clarify this problem.

The results obtained after the intraperitoneal injections of labelled 3,4,5-TMPAA showed (Table 4) that only a small amount if any of this acid in brain, after i.p. injection of mescaline, is of peripheral origin, since the proportion of 3,4,5-TMPAA penetrating from blood into brain is small.

Still better evidence was obtained for the oxidative metabolism of mescaline, and the formation of 3,4,5-TMPAA in brain by intraventricular mescaline injections. Despite chloralhydrate narcosis, the elimination rate of mescaline and the concentration profile of 3,4,5-TMPAA in brain was practically identical with that observed after intraperitoneal injection of mescaline, although the brain–blood ratios differed very considerably in the two experiments (Tables 3 and 5). The inhibition of 3,4,5-TMPAA outflow by Probenecid showed finally the *in situ* formation of acidic metabolites from mescaline. One can appraise from the data of Fig. 2 and Tables 1 and 2, that only about 10 per cent of the mescaline is eliminated from brain after its oxidative degradation.

The concentrations of mescaline and of its metabolites in different brain regions (Fig. 3) were largely those expected as derived from the studies on whole brain. Metabolite concentrations were always lower than amine concentrations, although

the amine–metabolite ratios were somewhat lower than in whole brain. Inversely, in the case of 2,3,4-TMPEA, the radioactivity present in the different brain regions consisted nearly exclusively of metabolites (Fig. 4).

The highest initial amine concentrations were observed in the hypothalamus, supposedly because of its high capillarization, and its contact with CSF. The highest initial elimination rates of amine were observed in this region, probably due to the high capacity of this tissue to oxidize mescaline and 2,3,4-TMPEA[24] and to its high capillarization. After the initial steep decline of radioactivity in the hypothalamus, elimination of mescaline slowed down, the elimination curve showing a constant mescaline concentration at 6 hr. The elimination curve in the thalamus only resembled that of the hypothalamus, although the initial amine concentrations were considerably lower (Fig. 3). The decline of 3,4,5-TMPAA concentration in hypothalamus was, however, comparable to that in other regions.

The regional distribution of mescaline in mouse brain correlates with that of radioactive LSD-25, which has been localized in the brain of monkeys preferentially in the cortical structures of the limbic system and in the basal ganglia.[39]

Previous autoradiographic studies on the distribution of radioactivity in mouse brain after mescaline administration[26,27] showed almost the same distribution pattern at 1 and 3 hr. However 6 hr after mescaline administration a different distribution was observed in the hippocampus than that presumed from biochemical studies. Radioactivity seemed to persist in this structure of the limbic system more selectively than in any other brain region over a long time period. On the other hand an action of mescaline in the limbic system seemed to be probable.[40–43] We have no explanation for the apparent discrepancy observed in the regional distribution of mescaline and its metabolites with autoradiographic and biochemical methods. It should however be noted that the selective labelling of the hippocampus after the administration of several hallucinogens and of compounds, which antagonize hallucinations has been reported by several groups.[28–35] The unique localization in the hippocampus of mescaline and of other hallucinogens is therefore presumably connected to certain methodological factors of light microscopic autoradiography.

Binding of mescaline to proteins *in vivo* was postulated more than 20 years ago, by Block *et al.*[12,43,44] Although binding phenomena were only briefly mentioned in the present work, we nevertheless want to make some remarks concerned with our findings, because we agree with Block, that the formation of homeopolar bonds of mescaline or of a mescaline metabolite with a certain "receptor" could be a key reaction for the hallucinogenic activity of mescaline, although we have no evidence for this. Radioactivity of the acid insoluble tissue constituents was not proportional to the total radioactivity in brain; and since the relative content of mescaline in comparison to mescaline metabolites was diminished (Table 1) with increasing incorporation time, it seems that the time dependent relative increase of radioactivity of the acid insoluble tissue constituents (Fig. 1) is significant, and not due to spontaneous incorporation into the macromolecular fraction during tissue processing.[45] The diminuition of mescaline derived insoluble radioactivity by inhibition of MAO with Pargyline is in accordance with the observations of Alivisatos *et al.*[46] with serotonin. However, we are not convinced that the oxidative degradation of mescaline is a necessary step before binding to macromolecules occur. Another explanation, of our *in vivo* experiments at least, is the known increase in the concentration of endogenous

amines, which may compete with mescaline or its metabolites for the receptor. Increase of mescaline concentration in brain by semicarbazide pretreatment, without inhibition of MAO, resulted in increased binding of mescaline derived radioactivity, without at the same time increasing the acidic mescaline metabolite concentration in brain. This observation is in agreement with the suggestion of competitive inhibition of binding of amines to macromolecules. The increased radioactivity content of the acid insoluble tissue constituents in Pargyline treated animals 3 hr after the administration of [^{3}H]2,3,4-TMPEA, and the lack of this Pargyline effect at 6 hr (Fig. 1), also seems to support this view, since the concentration of the endogenous amines increases with time, while the concentration of 2,3,4-TMPEA in brain decreases gradually, despite of MAO inhibition.

The data of Smythies *et al.*[7] provide evidence that the psychotomimetic properties of methoxy-β-phenylethylamines depend on the presence of three methoxy groups in the 3,4,5-configuration. Additional methoxy groups increase the activity. The corresponding amphetamines have normally more pronounced psychotomimetic activity,[47] however, the influence of the configuration of the methoxy-groups on the behavioral activity is not completely analogous between the β-phenylethylamines and the β-phenylpropylamines: 2,4,5-trimethoxy-amphetamine is some 17 times as active as mescaline, whereas the 2,4,5-trimethoxy-β-phenylethylamine is inactive.

One of the major differences between mescaline and its non-hallucinogenic isomer, 2,3,4-trimethoxy-β-phenylethylamine is their different susceptibility to oxidative degradation *in vitro*[24] and *in vivo*. With an enzyme preparation of rabbit liver it was shown[48] that the β-phenylethylamines with methoxy groups in 2 and 6 positions (2,6-, 2,3,6-, and 2,4,6-) and all compounds with more than 3 methoxy-groups were not oxidized. Immunity to attack by MAO is therefore clearly not sufficient condition for psychotomimetic activity, however, possibly one of the conditions which may be of some importance; the inertness of the amphetamines to degradation by MAO is at least in accordance with this assumption. It should be emphasized that certain behavioral effects of mescaline in mice, especially effects on motoric activity, are observed several hours after its application,[12,26] and numerous other observations[19,20] seem to indicate the necessity of the transformation of mescaline into an active form. Susceptibility to rapid oxidative degradation makes, however, the amine inaccessible to receptor sites, both for direct interaction, and more probably, for conversion into the active derivatives.

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