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THE METABOLISM OF MESCALINE-^{14}C IN RATS

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Abstract—The metabolism of mescaline-^{14}C and N-acetylmescaline-^{14}C was investigated in rats. N-Acetylmescaline, N-acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine and N-acetyl-3,5-dimethoxy-4)hydroxyphenylethylamine were isolated from the urine and identified as metabolites of mescaline. After the administration of N-acetylmescaline the major metabolites excreted in the urine of rats were N-acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine and N-acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine. Suggestive evidence is presented that N-acetylation of mescaline preceeds O-demethylation.

In iproniazid-treated animals the excretion of N-acetylated mescaline metabolites is increased, whereas the excretion of the deaminated metabolites is decreased as compared with the corresponding controls.

β-Hydroxymescaline was not detected as a metabolite after the administration of mescaline to the rat.

THE CAUSE of the hallucinogenic effects produced by mescaline is not yet well understood. It has been suggested[1] that a metabolite of mescaline may be the active agent and, therefore, an extensive study of this amine is warranted. Studies *in vivo* have shown that a large part of mescaline combines with liver protein[1] and that the amine is excreted unchanged or as the oxidatively deaminated product 3,4,5-trimethoxyphenylacetic acid.[2] It has also been shown that 3,4,5-trimethoxyphenylethanol is a metabolite of mescaline in the rat.[3] As minor metabolites, 3,4-dihydroxy-5-methoxyphenylacetic acid[2] and 3,4-dimethoxy-5-hydroxyphenylethylamine were isolated from human urine.[4] The formation *in vitro* of 3,4-dimethoxy-5-hydroxyphenylethylamine and 3,5-dimethoxy-4-hydroxyphenylethylamine from mescaline was reported.[5] Goldstein and Contrera[6] have shown the mescaline is a weak substrate for dopamine-β-hydroxylase, but did not establish whether this drug undergoes β-hydroxylation *in vivo*. Biogenic amines undergo N-acetylation *in vivo*[7, 8] and it seems possible, therefore, that mescaline is also metabolized by this pathway.

The present report explores the metabolic pathways of mescaline *in vivo* and elucidates whether and to what extent β-hydroxylation, N-acetylation, and O-demethylation occur *in vivo*. Preliminary reports of these studies have been presented.[7, 9]

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METHODS

Male Sprague–Dawley rats weighing 250–300 g were placed in groups of two in plastic metabolic cages. Iproniazid (100 mg/kg) was given i.p. 16 and 3 hr prior to the i.p. injection (0·2 mg/kg) of mescaline-8-^{14}C (sp. act. 2·19 mc/m-mole). In some experiments the animals received 0·2 mg mescaline-^{14}C/kg and 40 mg nonradioactive mescaline/kg. In other experiments rats were treated (i.p.) with N-acetyl mescaline-^{14}C ($4{\cdot}8 \times 10^6$ cpm per rat) or with N-acetyl mescaline-^{14}C in combination with nonradioactive N-acetyl mescaline (40 mg and 2×10^7 cpm per kg i.p.). After these treatments the rats were fasted, but water was offered *ad libitum*, and the urine was collected for a period of 24 hr.

N-Acetyl mescaline and N-acetyl mescaline-^{14}C were prepared by acetylation of the corresponding amine as previously described.[7] 3,4-Dimethoxy-5-hydroxyphenylethylamine was kindly donated by Dr. Peter Smith; 3,5-dimethoxy-4-hydroxyphenylethylamine was synthesized by condensation of 3,5-dimethoxy-4-hydroxybenzaldehyde with nitromethane followed by lithium aluminum hydride reduction.[10] The purity of the synthesized compound was established by paper chromatography in two different solvent systems. N-Acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine and N-acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine were prepared by acetylation of the corresponding amines followed by the hydrolysis of the O-acetyl group (by heating at 100° for 5 min in 2% sodium carbonate). β-Hydroxy mescaline was synthesized by condensation of 3,4,5-trimethoxybenazldehyde with potassium cyanide, and subsequently the cyanohydrin was reduced with $LiAlH_4$.[11] The product was then recrystallized from ethanol–ether as white needles, m.p. 200–203° uncorr. (m.p. lit. 196–199°).

The 24-hr urine was diluted up to 100 ml, and aliquot was adjusted to pH 1 with HCl and hydrolyzed by refluxing at 100° for 30 min. After cooling, the urine was extracted four times with an equal volume of ethyl acetate. The aqueous phase contained the amine fraction; the organic phase contained the acidic and neutral metabolites.

Isolation of the amines. An aliquot of the aqueous phase was adjusted to pH 8·5 with N NaOH and extracted twice with three volumes of *n*-butanol. To the pooled *n*-butanol extract an equal volume of heptane was added. One hundred ml of the organic mixture was shaken with 15 ml of 0·1 N HCl, and the total radioactivity of the amines was determined by counting aliquots of the aqueous layer. In control experiments it was established that the overall recovery of mescaline by this procedure was about 65 per cent. Corrections were made for recovery and for quenching. The aqueous phase containing the amines was then lyophilized, and the dry residue was washed three times with methanol. An aliquot of the methanol extract was subjected to paper chromatography in butanol:acetic acid:H_2O (4:1:5), and in isopropyl alcohol:amonia:water (4:1:1) systems.

Isolation of neutral and acidic metabolites. The ethyl acetate extract was submitted to descending paper chromatography, with benzene-acetic acid-water (2:1:1) as a solvent system. The chromatograms were scanned, and radioactive zones were detected with R_f's of 0·37; 0·49; 0·74; 0·90. One radioactive zone that remained at the origin was not identified. Each radioactive zone was eluted from the paper by extraction three times with 10 ml methanol. Aliquots of the eluates were subjected to paper chromatography in two other solvent systems.

Adsorption on alumina and on Dowex 50W-X4 ion-exchange resin. Aliquots of the eluates obtained from the radioactive zones after paper chromatography were evaporated under nitrogen to dryness, and the residues were dissolved in 10 ml of 0·1% methanol in water and passed at pH 8·5 through an alumina column. The adsorbed catechols were eluted with 6 ml of 0·2 N acetic acid. The effluents and the washings from the alumina columns were adjusted to pH 5·0 and passed through a column (6 × 20 mm) of Dowex 50W-X4 ion-exchange resin in the H^+ form. The resin column was washed with 15 ml of glass-distilled water, and the amines were eluted with 20 ml of N ammonium hydroxide in 65% ethanol. Aliquots of the eluates and of the effluents from the alumina and Dowex columns were evaporated to dryness in vials and the radioactivity estimated by means of scintillation counting.

Alkali hydrolysis. Aliquots of the eluates of the radioactive zones obtained by paper chromatography of the ethyl acetate fraction were transferred to round-bottom flasks and dried under vacuum. The hydrolysis was carried out by addition of 10 ml of N potassium hydroxide and heating at 100° and refluxing during 2 hr under a nitrogen atmosphere. After cooling, the pH was adjusted to 5 with 0·5 N perchloric acid and left overnight at 4°. The potassium perchlorate crystals were removed by filtration and the filtrate passed through a Dowex 50W-X4 (H^+ form) ion-exchange resin column (4 × 0·8 cm). The amines were eluted from the column as described above. The eluate was concentrated under vacuum, and aliquots were applied on Whatman paper No. 1 and chromatographed in two different solvent systems.

RESULTS

Amines

Identification of mescaline. On paper chromatography, in two solvent systems, the amine fraction showed one radioactive zone that had the same R_f value as authentic mescaline (*n*-butanol:acetic acid:water, 4:1:1), R_f 0·65; the other, isopropyl alcohol: ammonia:water (8:1:1) R_f 0·80). Upon acetylation of the amine fraction the radioactive material had the same paper chromatographic characteristics as authentic N-acetyl mescaline (Table 1).

Neutral and acidic metabolites

Identification of N-acetyl 3,5-dimethoxy-4-hydroxyphenylethylamine (Compound I). The compound obtained from the slowest moving radioactive zone (R_f 0·37) coincided with authentic N-acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine (Compound I, Fig. 1) in two different chromatographic solvent systems (Table 1). The radioactive material was not adsorbed on aluminum oxide at pH 8·5 nor on Dowex 50W-X4 ion-exchange resin (H^+ form).

After the administration of mescaline-^{14}C in combination with nonradioactive mescaline, the radioactive material isolated from the urine showed characteristic color reactions of N-acetyl 3,5-dimethoxy-4-hydroxyphenylethylamine upon spraying with phenolic reagents (Table 1). These color reactions were observed only when the rats were treated with a large dosage of nonradioactive mescaline.

Further evidence of the identity of Compound I was obtained by alkaline hydrolysis of the N-acetyl group. Upon hydrolysis a radioactive compound with the same R_f value and characteristic color reactions as authentic 3,5-dimethoxy-4-hydroxyphenylethylamine was obtained (Table 1).

Identification of N-acetyl 3,4-*dimethoxy-5-hydroxyphenylethylamine* (*Compound II*). Compound II, Fig. 1, was identified by the same general procedure as that outlined for Compound I. The radioactive zone corresponding to Compound II had the same R_f value in two different chromatographic systems and gave the same color reactions

TABLE 1. CHARACTERIZATION DATA FOR MESCALINE METABOLITES

Metabolite	Derivative*	R_f in different solvents†				Color reactions		
		A	B	C	D	Sulfanilic acid	*p*-Nitro aniline	Gibb's
Compound I: N-acetyl 3,5-dimethoxy-4-hydroxyphenyl ethylamine		0·37	0·54			None	blue-gray	blue
	3,5-Dimethoxy-4-hydroxyphenyl-ethylamine			0·46	0·56	pink	blue-gray	blue-green
Compound II: N-acetyl 3,4-dimethoxy-5-hydroxyphenyl-ethylamine		0·49	0·71			brown	dark red	light blue
	3,4-Dimethoxy-5-hydroxyphenyl-ethylamine			0·59	0·63	orange	dark-red	light blue
Compound III: N-acetyl mescaline		0·74		0·85	0·90			
	Mescaline			0·66	0·70			

* The N-deacetylated derivatives were obtained upon alkali hydrolysis and purified on Dowex 50 × 2 column.

† A: Benzene:acetic acid:water (2:1:1). B: Toluene:ethylacetate:methanol:water (1:1:1:1). C: *n*-Butanol:acetic acid:water (4:1:1). D: Isopropyl alcohol:ammonia:water (8:1:1).

with phenolic reagents as authentic N-acetyl 3,4-dimethoxy-5-hydroxyphenylethylamine (Table 1). The radioactive material was not adsorbed on aluminum oxide at pH 8·6 nor on Dowex 50W-X4 ion-exchange resin. On alkaline hydrolysis, an amine was formed with the same ion-exchange and paper chromatographic characteristics as authentic 3,4-dimethoxy-5-hydroxyphenylethylamine.

Identification of N-acetylmescaline (*Compound III*). The mobility of the third radioactive zone in three different chromatographic solvent systems was identical with that of authentic N-acetylmescaline (Compound III, Fig. 1). The material present in this zone had shown no color reaction after spraying with phenolic reagents or with ninhydrin. It was not adsorbed on alumina nor on Dowex 50W-X4 ion-exchange resin. Upon hydrolysis of the radioactive material with N NaOH at 100° for 2 hr, an amine was recovered with the same chromatographic behavior as authentic mescaline.

Trimethoxyphenylacetic acid. The fastest moving radioactive zone in butanol: acetic acid: water (2-1-1), had the same R_f value as authentic trimethoxyphenylacetic

acid (0·90). The radioactive material had the same R_f value as trimethoxyphenylacetic acid in two different chromatographic solvent systems.

Metabolism of N-acetylmescaline-^{14}C

The data in Table 2 show that N-acetylmescaline-^{14}C is almost completely demethylated *in vivo* in the 4- or 5-position of the benzene ring. It is also shown that

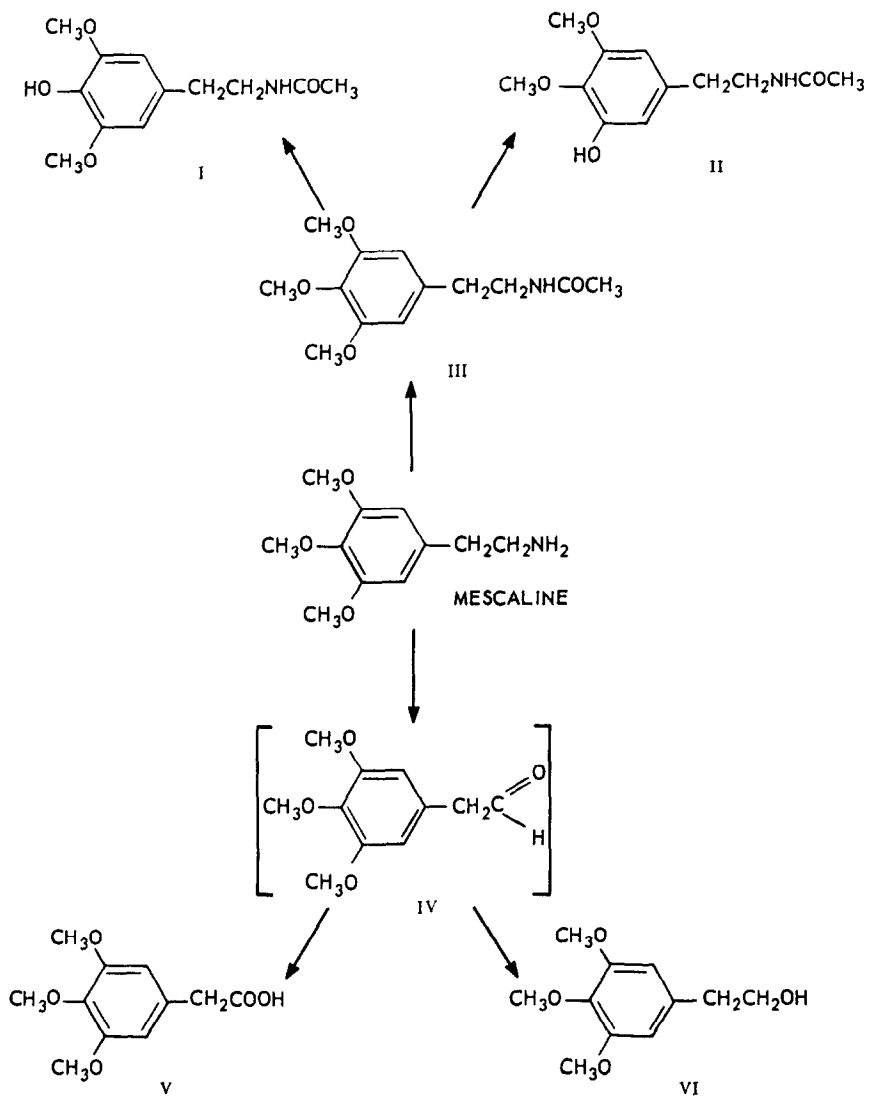

FIG. 1. Metabolic pathways of mescaline-^{14}C in the rat. Compound I = N-acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine; II = N-acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine; III = N-acetylmescaline; IV = 3,4,5-trimethoxyphenylacetaldehyde; V = trimethoxyphenylacetic acid; VI = trimethoxyphenylethanol.

50 per cent of the total radioactivity was excreted as N-acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine and 30 per cent as N-acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine. Mescaline-^{14}C was not detected in these urine samples. After the administration of N-acetylmescaline-^{14}C in combination with nonradioactive N-acetylmescaline, it was possible to identify compounds I and II by the typical color reactions (Table 1).

TABLE 2. METABOLITES OF N-ACETYLMESCALINE IN URINE OF RATS

	In 24-hr Urine (counts/min)	Total radioactivity excreted (%)
N-Acetylmescaline	154,000	5·4
N-Acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine	1,512,000	53·0
N-Acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine	798,000	28·0
Unknown	387,500	13·6
Total	2,851,500	100·0

Rats were treated with 4·8 × 10^6 counts/min of N-acetylmescaline-^{14}C. The results are the average of two experiments. The excretion pattern was the same when the rats received N-acetylmescaline-^{14}C plus 20 mg i.p. of N-acetylmescaline/kg.

The effects of iproniazid and pargyline on the excretion of mescaline-8-^{14}C and its metabolites

It is evident from the results presented in Table 3 that the metabolic pathway of mescaline is altered in iproniazid-treated animals. The metabolism by the N-acetylation pathway is enhanced, and the metabolism by deamination is inhibited. In pargyline-treated animals the metabolism of mescaline is qualitatively altered in the same manner as in the iproniazid-treated animals. However, in these animals, the metabolism by N-acetylation is not so much enhanced and the metabolism by deamination is not so much inhibited as in the iproniazid-treated animals.

DISCUSSION

The present study reveals that mescaline undergoes N-acetylation and O-demethylation *in vivo*. In addition to N-acetylmescaline, we have identified two other N-acetylated metabolites of mescaline, namely, N-acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine and N-acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine (Fig. 1). The formation of N-acetylated biogenic amines *in vivo* was previously described.[7, 8] N-acetylation of biogenic amines is enhanced after MAO inhibition. The present study shows that the excretion of N-acetylated mescaline metabolites is increased, whereas the excretion of the deaminated metabolites is decreased, after iproniazid administration. This is consistent with the report that mescaline is a substrate of diamine oxidase and that iproniazid is an inhibitor of this enzyme.[12]

The formation of acetylated metabolites may yield compounds that are biologically more active than the parent compound (i.e. acetylcholine, melatonin) or metabolites that are biologically inactive (i.e. N-acetyl serotonin, N-acetyl dopamine, N-acetyl

norepinephrine). N-Acetylmescaline produced no behavioral effects in rats given a dosage even as high as 40 mg/kg (Chorover, personal communication). In humans also, N-acetylmescaline produces no physiological or behavioral effects.[13] It seems, therefore, that N-acetylation of mescaline represents an inactivation process.

TABLE 3. MESCALINE-^{14}C METABOLITES IN URINE OF UNTREATED AND IPRONIAZID-TREATED RATS

	Treatment	
	None	Iproniazid
Mescaline	20·1 ± 3·7	43·10 ± 6·40
Trimethoxyphenylacetic acid*	42·3 ± 5·3	1·45 ± 0·35
N-Acetylmescaline	1·7 ± 0·2	5·55 ± 0·75
N-Acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine	15·1 ± 2·9	27·75 ± 3·55
N-Acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine	14·4 ± 1·7	16·85 ± 1·45
Unknown	6·4 ± 1·0	5·30 ± 0·30

Results are the average of three experiments and are expressed as the percentage ± S.E.M. of the total radioactivity excreted during 24 hr after the injection of mescaline-8-^{14}C.

* Radioactivity corresponding to trimethoxyphenylethanol was not separated.

The N-acetylated metabolites of mescaline were demethylated in positions 4 and 5 of the benzene ring. Neither O-demethylated mescaline nor O-demethylated acidic metabolites of mescaline were detected in the urine of rats. This finding indicates that N-acetylation precedes O-demethylation. Also, the finding that N-acetylmescaline undergoes O-demethylation to a great extent *in vivo* gives further support to the hypothesis that N-acetylation precedes O-demethylation.[9] It has been reported that in man O-demethylation of mescaline[4] and of trimethoxyphenylacetic acid[13] occurs to a small extent. Thus, N-acetylation and O-demethylation of mescaline represent a metabolic pathway in man as well as in animals.

The finding that N-acetylmescaline undergoes more extensive dealkylation than mescaline or trimethoxyphenylacetic acid might be explained on the basis of the greater penetration of N-acetyl derivatives into the microsomes where the demethylating enzymes are localized. It is also noteworthy that N-acetylmescaline is not deaminated, and therefore O-demethylation represents the only process by which the compound becomes hydrophilic and can then by excreted in the free or conjugated form.

3,4,5-Trimethoxyphenylacetic acid has been identified as a metabolite of mescaline in the urine of man, mice, and rats.[2, 14] It has recently been reported that in cats the only metabolite of mescaline is 3,4,5,-trimethoxyphenylacetic acid and that no demethylated or N-acetylated metabolites of mescaline were detected.[15] However, studies in our laboratory have shown that mescaline also undergoes N-acetylation and O-demethylation in the cat, amounting to 2 per cent of the total excreted radioactivity (Musacchio and Goldstein, unpublished data).

The β-side-chain hydroxylation of phenylethylamines usually yields products that are pharmacologically more active than the parent compound, i.e. conversion of dopamine to norepinephrine, tyramine to octopamine, etc. The psychopharmacological activity of β-hydroxymescaline was compared with mescaline in cats. β-Hydroxymescaline shows slight transient signs of sympathomimetic stimulation at 16 mg/kg. However, no evidence of the bizarre behavior noted with mescaline after doses of approximately 4 mg/kg was observed (Irwin, personal communication). These findings, as well as the failure to detect β-hydroxymescaline in rat urine after administration of mescaline, excludes the possibility that the behavioral effects of mescaline in animals are mediated via its β-hydroxylated product.

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