Pharmacology *13*: 273–280 (1975)

Studies on Accumulation of (^{14}C)-Mescaline in Brain Homogenates: Effects of Psychotropic and Other Agents[1]

Nandkumar S. Shah and O.D. Gulati

Ensor Foundation Research Laboratory, William S. Hall Psychiatric Institute, Columbia, S.C.

Key Words. Accumulation · Mescaline · Phenothiazines · Rat brain homogenate · Tricyclic antidepressants

Abstract. Incubation of rat brain homogenates or 14,500 *g* pellet isolated from the homogenate with (^{14}C)-mescaline was associated with accumulation of (^{14}C)-mescaline in the pellet. 1.33 μmol/ml of chlorpromazine, trifluoperazine, fluphenazine, imipramine, desmethylimipramine, nortriptyline and amitriptyline inhibited the accumulation of mescaline. Lower concentrations (0.133–0.44 μmol/ml) of the psychotropic drugs were less effective. The tricyclic antidepressants were less potent than the tranquilizers. Although the trimethoxyphenylacetic acid (TMPA) levels of the pellet were also reduced by the psychotropic drugs, the TMPA:mescaline ratios were unchanged indicating that the drugs had no effect on the metabolism of mescaline. The inhibition of accumulation of mescaline by the high concentrations of tranquilizers may divert more of the hallucinogen to the receptor site. Thus, an explanation for the reported worsening of clinical syndrome of hallucinogenic poisoning by tranquilizers is provided.

Shah and Himwich (13) examined the accumulation of mescaline by mouse and rat brain crude mitochondria and found that small amounts of this amine were accumulated by a process of simple binding. Further, the accumulation by crude brain mitochondrial preparations of labeled dopamine, a compound structurally similar to mescaline, is profoundly inhibited by chlorpromazine and other phenothiazines (10). The present investigation reports the effects of various psychotropic drugs, some catecholamines, indoleamines and a few nucleotides on the accumulation of mescaline in the rat brain homogenate.

[1] This work was presented at the Fall Meeting of the American Society of Pharmacology and Experimental Therapeutics in Montreal, Canada, August 1974.

Received: January 7, 1975.

Materials and Methods

Chemicals. 8-(^{14}C)-mescaline hydrochloride (spec. act. 4.53 mCi/mmol) was purchased from New England Nuclear Corp. (Boston, Mass.). The chemical and radiochemical purity was ascertained in our laboratory by column and thin-layer chromatography and found to be 97 %. Chlorpromazine hydrochloride, trifluoperazine dihydrochloride (Smith, Kline & French Laboratories, Philadelphia, Pa.), imipramine hydrochloride, desmethylimipramine hydrochloride (Geigy Pharmaceuticals, Ardsley, N.Y.), fluphenazine dihydrochloride (Squibb, New Brunswick, N.J.), nortriptyline hydrochloride (Eli Lilly & Co., Indianapolis, Ind.) and amitriptyline hydrochloride (Merck Sharp & Dohme, Rahway, N.J.) were received as generous gifts. All other chemicals purchased from commercial sources were of high purity.

Accumulation of (^{14}C)-mescaline by brain homogenate. Male Sprague-Dawley rats (170–200 g) were decapitated and the brains were quickly removed. The brain minus the cerebellum was homogenized in 10 vol of ice-cold 0.25 *M* sucrose solution using glass homogenizer. All further procedures prior to incubation were carried out in the cold. The homogenates were centrifugated at 2,100 *g* for 10 min to remove unbroken tissue fragments and other debris. The protein content of the supernatant was determined by the biuret reaction according to *Hussain et al.* (7). A 1.0-ml aliquot equivalent to 10 mg of protein was added to 1.0 ml of 0.1 *M* Tris-HCl buffer (pH 7.5), 0.2 ml of 2.5 *M* sodium chloride solution and 0.5 ml of 0.25 *M* sucrose solution in a 25-ml Erlenmeyer flask. The compounds under test were dissolved in 0.5 ml of 0.25 *M* sucrose solution and added to flasks before incubation. After an equilibration period of 10 min at 37 °C in a Dubnoff metabolic shaker in an atmosphere of air, a 0.3-ml solution of (^{14}C)-mescaline containing 0.5 μCi was added and the incubation was continued for an additional 30 min. The samples were then removed to an ice bath and transferred to centrifuge tubes. After centrifugation at 14,500 *g* for 15 min at 4 °C, the resulting pellet was rinsed twice with 3.0 ml of 0.25 *M* sucrose and extracted successively with 5.0 and 3.0 ml of cold 0.4 *N* perchloric acid (14). The extracts were adjusted to pH 5.8 with 5 *N* potassium carbonate solution. The unchanged (^{14}C)-mescaline was separated from the principal deaminated metabolite, 3,4,5-trimethoxyphenylacetic acid (TMPA) according to the procedure outlined by *Charalampous et al.* (2) as modified by *Shah and Himwich* (12). The overall recovery through the isolation procedure ranged from 74 to 86 %. A 1.0-ml aliquot of each of unchanged mescaline or TMPA was added to 10 ml scintillation fluid (1) and the radioactivity was determined in a Nuclear Chicago Mark II liquid scintillation counter using ^{133}Ba as an external standard. The efficiency of the counting system ranged between 83 and 88 %. All data were corrected for counting efficiency and for the recovery.

Accumulation of (^{14}C)-mescaline by isolated pellet. Brain homogenates were centrifuged at 2,100 *g* and protein concentration of the supernatant was determined as described above. The supernatant was centrifuged at 14,500 *g* for 15 min. The pellet was washed twice with 3.0 ml of 0.25 *M* sucrose solution and finally resuspended in the sucrose solution. A 1.0-ml pellet suspension (equivalent to 10 mg of protein of the original homogenate) was then incubated with various drugs in a manner similar to that described for the homogenate.

In another set of experiments, the effect of high temperature on the ability of particles to accumulate mescaline was investigated. Aliquots of particle suspension were left at 56 °C for 30 min and returned to ice for 10 min. The preparation was subsequently used for determining mescaline accumulation as described above.

Statistics. The levels of (^{14}C)-mescaline and of (^{14}C)-TMPA were calculated as the mean ± SD. The statistical significance of differences between mean values was determined with a two-tailed Student's t test and p value of 0.05 or less was considered significant.

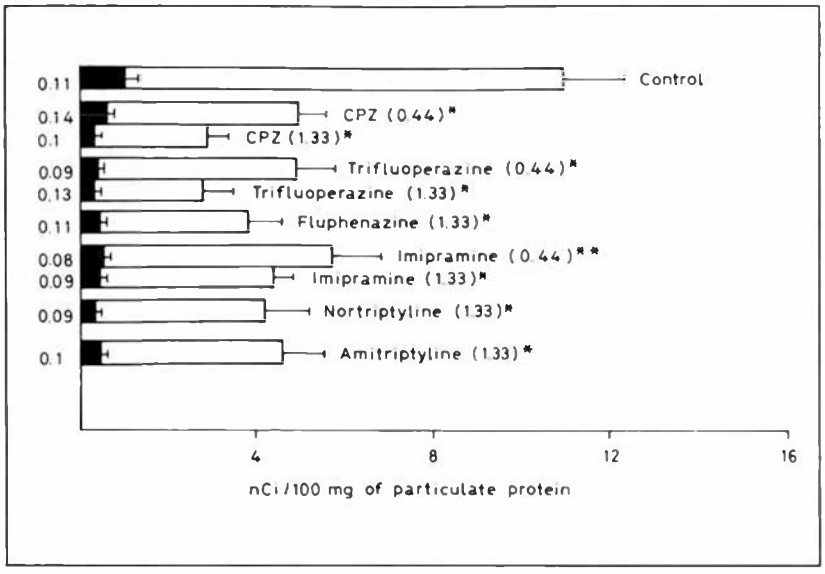

Fig. 1. Rat brain homogenates incubated at 37 °C for 30 min with (^{14}C)-mescaline (0.5 μCi/3 ml) in the presence and absence of various psychotropic drugs. The levels of (^{14}C)-mescaline (stippled bars) and of (^{14}C)-trimethoxyphenylacetic acid (TMPA, white bars) are expressed as nCi/100 mg of particulate protein. The figures in parentheses to the right of each bar represent the concentration of the psychotropic drug (μmol/ml incubation medium) and the figures to the left of each bar represent the TMPA:mescaline ratios. Each bar represents the mean of four separate determinations run in duplicate and horizontal lines represent the SD. p values of the differences from the control mean mescaline concentrations are depicted by * (< 0.001) and ** (< 0.005). CPZ = Chlorpromazine. ■ = (^{14}C) TMPA; □ = (^{14}C) mescaline.

Results

Mescaline accumulation in the homogenate. When brain homogenates were incubated at 37 °C for 30 min with (^{14}C)-mescaline, there was a small accumulation of radioactivity in particles which could subsequently be recovered by centrifugation. The amounts of unchanged (^{14}C)-mescaline and of (^{14}C)-TMPA in particles were 9.41 ± 1.37 and 1.07 ± 0.28 nCi/100 mg of particulate protein, respectively (fig. 1). Imipramine (1.33 μmol/ml) reduced the levels of mescaline to about 43 % ($p < 0.001$). Amitriptyline and nortriptyline were as active as imipramine whereas chlorpromazine, trifluoperazine and fluphenazine in the same concentration were about 2 times more effective than imipramine. When the concentrations of chlorpromazine, trifluoperazine and imipramine in the incubation media were reduced to 0.44 μmol/ml, the levels of mescaline in the

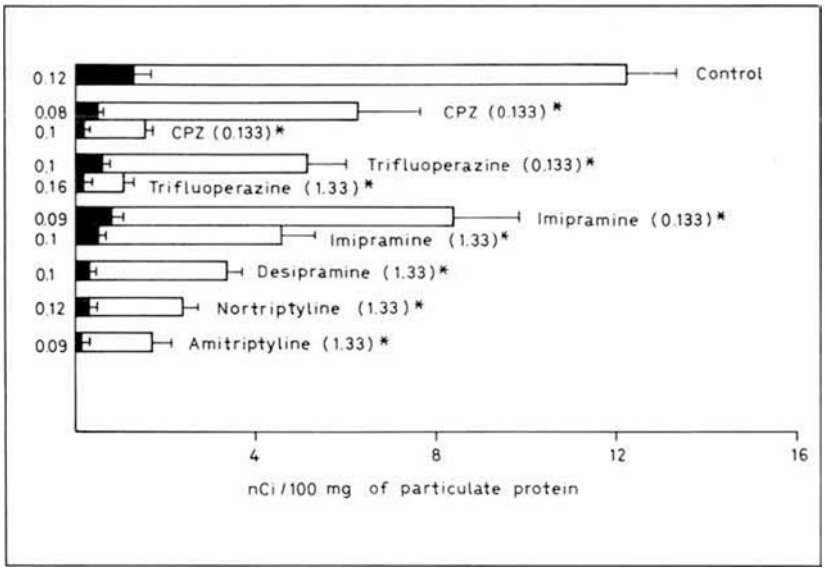

Fig. 2. Pellets of rat brain homogenates incubated at 37 °C for 30 min with (^{14}C)-mescaline (0.5 μCi/3 ml) in the presence and absence of various psychotropic drugs. The levels of (^{14}C)-mescaline (stippled bars) and of (^{14}C)-trimethoxyphenylacetic acid (TMPA, white bars) are expressed as nCi/100 mg of particulate protein. The figures in parentheses to the right of each bar represent the concentration of the psychotropic drug (μmol/ml incubation medium) and the figures to the left of each bar represent the TMPA:mescaline ratio. Each bar represents the mean of 4–12 separate determinations run in duplicate and horizontal bars represent the SD. p values of the differences from the control mean mescaline concentration are depicted by * (< 0.001). CPZ = Chlorpromazine. ■ = (^{14}C)-TMPA; □ = (^{14}C)-mescaline.

pellet were still 40–60 % of those of controls. While the total amounts of TMPA were decreased, the ratios TMPA:mescaline remained unaltered by the addition of various drugs indicating that the psychotropic drugs do not interfere with the conversion of mescaline to TMPA.

Mescaline accumulation in the pellet. The control levels of (^{14}C)-mescaline and (^{14}C)-TMPA in particles were 10.9 ± 1.1 and 1.34 ± 0.28 nCi/100 mg protein, respectively (fig. 2). These levels were virtually identical to those observed in particles (fig. 1) isolated from homogenate incubated with mescaline. Imipramine, amitriptyline and nortriptyline (1.33 μmol/ml) markedly reduced the accumulation of labeled mescaline. Chlorpromazine and trifluoperazine were about 3 times more effective than imipramine as inhibitors of mescaline accumulation. Although desmethylimipramine was somewhat more potent than imipramine, it was half as active as chlorpromazine or trifluoperazine. In other

Table I. Effect of some drugs on the accumulation of (^{14}C)-mescaline in 14,500 *g* pellet pretreated at 56 °C

Test compound μmol/ml	(^{14}C)-mescaline mean nCi/100 mg of particulate protein ± SD	Change from control %
Control (no test compound)	3.33 ± 0.51 (4)	–
Chlorpromazine (1.33)	1.77 ± 0.20 (4)	53.2
Nortriptyline (1.33)	2.97 ± 0.59 (4)	89.2
Imipramine (1.33)	2.37 ± 0.36 (4)	71.2

Particulate fraction (14,500 *g* pellet) isolated from brain homogenate was incubated at 56 °C for 30 min in a Dubnoff metabolic shaker; the preparation was returned to ice for 10 min. A 1.0-ml aliquot was incubated at 37 °C for 10 min in a medium containing Tris-HCl buffer (pH 7.5), NaCl and the compounds listed above. Radiolabeled (^{14}C)-mescaline (0.5 μCi/3 ml) was added to each flask and incubation continued for an additional period of 30 min. Figures in parentheses in the second column indicate the number of experiments.

Table II. Lack of effect of some compounds on the accumulation of (^{14}C)-mescaline by the 14,500 *g* pellet isolated from rat brain homogenate

Test compound μmol/ml	(^{14}C)-mescaline mean nCi/100 mg of particulate protein ± SD	Change from control %
Control (no test compound)	11.5 ± 1.9 (6)	–
5-Methoxy-tryptamine (1.33)	10.3 ± 1.5 (4)	89.6
N-Acetyl-serotonin (1.33)	12.8 ± 2.6 (4)	111.3
DMPEA (1.33)	10.6 ± 0.74 (4)	92.2
L-Dopa (1.33)	11.7 ± 1.44 (4)	101.7
Dopamine (1.33)	11.2 ± 0.21 (4)	97.4
dl-Norepinephrine (1.33)	11.1 ± 0.18 (4)	96.5
ATP (2.25)	11.0 ± 1.3 (4)	95.7
ADP (2.25)	11.4 ± 0.8 (4)	99.1
AMP (2.25)	13.4 ± 1.6 (4)	116.5

A particulate fraction (14,500 *g* pellet) isolated from the brain homogenate was incubated at 37 °C for 10 min in a medium containing Tris-HCl buffer (pH 7.5), NaCl and the compounds listed above. Radiolabeled (^{14}C)-mescaline (0.5 μCi/3 ml) was added to each flask and incubation continued for an additional period of 30 min. Figures in parentheses in the second column indicate the number of experiments.

experiments the concentration of the inhibitors was reduced to one tenth (0.133 μmol/ml). Both chlorpromazine and trifluoperazine still caused significant inhibition of mescaline accumulation but the inhibition was less marked. Imipramine at a concentration of 0.133 μmol/ml was considerably less effective than the phenothiazines. The total amounts of TMPA were decreased by the various drugs, but the ratios of TMPA:mescaline remained unaltered.

In particle suspension exposed to 56 °C for 30 min and subsequently incubated with mescaline, the control mescaline content was 3.33 ± 0.51 nCi/100 mg protein (table I). Chlorpromazine (1.33 μmol/ml) lowered mescaline levels to about 50 % of the control; both imipramine and nortriptyline had only a slight effect on the accumulation.

Several compounds of pharmacological interest were tested for their effects on mescaline accumulation (table II). In a concentration of 1.33 μmol/ml, *dl*-norepinephrine, dopamine, *L*-dopa, 3,4-dimethoxyphenylethylamine (DMPEA), N-acetylserotonin and 5-methoxytryptamine, rendered no significant effect. Likewise, ATP, ADP and AMP (2.25 μmol/ml) were without effect.

Discussion

The accumulation of mescaline by the rat brain homogenate has been examined. A 14,500 *g* pellet which contains crude mitochondrial preparation accumulated mescaline. That the particulate preparation was viable was indicated by a considerable reduction of accumulation when the particles exposed to 56 °C for 30 min were incubated with mescaline. Previous *in vivo* studies (9) have demonstrated that the mitochondria isolated from mouse brain accumulate smaller amounts of mescaline than other subcellular fractions. From *in vitro* uptake studies, *Denber* (4) postulated that brain mitochondria probably have a fixed number of binding sites which are rapidly saturated with mescaline and that this condition is not changed even when additional amounts of mescaline are made available. In the present study a concentration of 167 nCi/ml of (^{14}C)-mescaline in the incubation medium resulted in a particulate accumulation of 94–109 nCi/g protein giving an accumulation ratio of less than unity. This observation coupled with the lack of stimulatory effect of ATP would militate against the uptake being a carrier-mediated active process. Indirect support for this conclusion is derived from the lack of inhibitory effect of DMPEA which differs from mescaline by the absence of a methoxy group on position 5 of the phenylethyl ring. Finally, the accumulation was not affected by NE and dopamine for which distinct neuronal uptake sites have been described (3). This conclusion is in accord with the previously reported lack of effect of ouabain and dinitrophenol on the accumulation of mescaline by the mouse and rat brain mitochondria (13).

The phenothiazines markedly reduced the accumulation of mescaline. The membrane stabilizing effects of phenothiazine (8) would preclude the membrane binding of mescaline and would suggest that the uptake, though not carrier-mediated, may be a form of facilitated diffusion. *Shah et al.* (15) demonstrated that chlorpromazine prolongs the disappearance of mescaline from the brain and several organs of mice. The membrane-stabilizing action of chlorpromazine invoked to explain the inhibition of influx of mescaline would also conceivably block the efflux of mescaline and account for the observations of *Shah et al.* (15).

There are clinical accounts of aggravation by chlorpromazine of the hallucinogenic effect of 4-methyl-2,5-dimethoxy-α-methylphenylethylamine, LSD and amphetamine (5, 16). *Halsaz et al.* (6) have shown that a tranquilizer such as chlorpromazine can act as a weak psychotogen protecting against LSD, a stronger hallucinogen, by substituting for it at receptor sites, but in large enough doses adding to or even producing the effect it was intended to correct and this could account for the reported occasional aggravation of hallucinogenic action by tranquilizers. These considerations led us to use rather high concentrations of the tranquilizers.

The results of the present study provide additional explanation for the clinically undesirable interactions reported between the tranquilizers and the hallucinogens. The phenothiazines, by preventing the accumulation of the hallucinogens (mescaline in the present case), may provide more of the hallucinogen to act at the receptor site and account for the occasional disturbing paradoxical effects.

Acknowledgments

This work was supported by the Ensor Research Foundation. The authors sincerely thank *Alexander G. Donald*, MD, Director, and *Joe E. Freed*, MD, Associate Director for Research and Training, William S. Hall Psychiatric Institute for their continued interest in this work.

References

1 *Bray, G.A.:* A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Analyt. Biochem. *1:* 279–285 (1960).

2 *Charalampous, K.D.; Walker, K.E., and Kinross-Wright, J.:* Metabolic fate of mescaline in man. Psychopharmacologia *1:* 48–63 (1966).

3 *Coyle, J.T. and Snyder, S.H.:* Catecholamine uptake by synaptosomes in homogenates of rat brain: stereospecificity in different areas. J. Pharmac. exp. Ther. *170:* 221–231 (1969).

4 *Denber, H.C.B.:* Intracellular localization of psychotomimetic and psychotropic drugs; dissertation Graduate School of Arts and Sciences, New York University (1967).
5 FDA: Science desk release, 2nd Aug., pp. 67–90 (1967).
6 *Halsaz, H.F.; Formanek, H.F., and Marrazzi, A.S.:* Hallucinogen tranquilizer interactions: its nature. Science *164:* 569–571 (1969).
7 *Hussain, Q.Z.; Shah, N.S., and Chaudhuri, S.N.:* Estimation of serum or plasma protein using qualitative Benedict reagent. Clinica chim. Acta *6:* 447–448 (1961).
8 *Seeman, P.:* The membrane actions of anesthetics and tranquilizers. Pharmac. Rev. *24:* 583–655 (1972).
9 *Shah, N.S.:* Subcellular distribution of 8-(^{14}C)-mescaline in the mouse brain and liver. Biochem. Pharmac. *20:* 3207–3210 (1971).
10 *Shah, N.S.:* Accumulation of ^{3}H-dopamine by isolated brain mitochondria. Brain Res. *38:* 391–398 (1972).
11 *Shah, N.S.:* Interaction of mescaline with antipsychotic drugs: comparative effects of chlorpromazine, thioridazine, fluphenazine and chlorprothixene on the disappearance of mescaline in mice. Soc. Biol. Psychiat. 29th Ann. Conv., p. 11 (1974).
12 *Shah, N.S. and Himwich, H.E.:* Study with mescaline-8-(^{14}C) in mice: effect of amine oxidase inhibitors on metabolism. Neuropharmacology *10:* 547–556 (1971).
13 *Shah, N.S. and Himwich, H.E.:* A comparative study of mescaline and 3,4-dimethoxyphenylethylamine in isolated brain mitochondria and brain homogenates. Brain Res. *34:* 163–170 (1971).
14 *Shah, N.S.; Kamano, A.; Glisson, S., and Callison, D.:* Studies on the uptake of radiolabeled dopa, 5-HTP and tryptophan in rat tissues *in vivo:* effect of methionine, tryptophan and some keto acids. Int. J. Neuropharmac. *7:* 75–86 (1968).
15 *Shah, N.S.; Shah, K.R.; Lawrence, R.S., and Neely, A.E.:* Effects of chlorpromazine and haloperidol on the disposition of mescaline-(^{14}C) in mice. J. Pharmac. exp. Ther. *186:* 297–304 (1973).
16 *Silverman, J.:* Acute schizophrenia: disease or dis-ease. Psychol. Today *4:* 62–65 (1970).

Dr. *Nandkumar S. Shah,* Ensor Research Laboratory, William S. Hall Psychiatric Institute, PO Box 119, *Columbia, SC 29202* (USA)