Biochemical Pharmacology, Vol. 20, pp. 3207–3210. Pergamon Press, 1971. Printed in Great Britain.

SHORT COMMUNICATIONS

Subcellular distribution of 8-^{14}C-mescaline in the mouse brain and liver*

(*Received* 11 *December* 1970; *accepted* 5 *March* 1971)

NUMEROUS reports have appeared concerning the absorption, regional localization and metabolism of mescaline into brain[1–3] but the information on distribution into various subcellular particles is lacking. Studies on the localization of lysergic acid diethylamide (LSD) in subcellular fractions obtained from hippocampus and frontal grey matters of monkey brain revealed a bulk of LSD in the soluble supernatant fraction.[4] This report is on the distribution of injected 8-^{14}C-mescaline into subcellular particles of brain and liver of control mice and of mice pretreated with iproniazid, semicarbazide or reserpine.

The 8-^{14}C-mescaline-hydrochloride (sp. act. 2·72 mc/m-mole) was purchased from New England Nuclear Corp. and semicarbazide-hydrochloride and mescaline-sulfate from Nutritional Biochemical Corp. Iproniazid phosphate and reserpine phosphate were received as gifts from F. Hoffmann La Roche Inc. and Ciba Foundation respectively.

Male albino mice, 35–40 g, were injected i.p. with 0·5 ml of a solution containing 2 μc of 8-^{14}C-mescaline-hydrochloride and 50 mg/kg of nonlabeled mescaline-sulfate calculated as free base. Iproniazid 200 mg/kg or semicarbazide 200 mg/kg was injected i.p. in 0·5 ml of a solution 4 hr prior to labeled mescaline; reserpine 2·5 mg/kg was given 18 hr prior to the beginning of experiments. The animals were individually housed in metabolic cages permitting the collection of urine at 1, 3, 8 and 24 hr. At selected times after mescaline, groups of mice were sacrificed by decapitation; the brains and livers were promptly removed and homogenized very gently in a hand-operated glass homogenizer containing 0·25 M chilled sucrose solution (pH 7·4). Homogenates were adjusted to 10% (w/v) by 0·25 M sucrose solution. A portion of the homogenate was set aside for the extraction and determination of total unchanged mescaline. The remaining portion was subjected to a differential centrifugation. The nuclear fraction was obtained by centrifugation for 10 min at 1000 *g* for the brain and 700 *g* for the liver. The resulting supernatant was saved; the pellet was resuspended in 2 ml of sucrose solution and centrifuged. The washing was combined with the first supernatant and then spun for 60 min at 105,000 *g*. The resulting supernatant was designated as soluble-supernatant; the pellet was resuspended in 2 ml of sucrose solution and centrifuged. The fluid portion was combined with soluble-supernatant. The pellet was designated the particulate fraction.

For the studies *in vitro* brains from untreated animals were removed and homogenized in chilled oxygenated Krebs–Ringer phosphate buffer containing glucose (pH 7·4). Two ml of a homogenate (10%, w/v) was placed in 25-ml Erlenmeyer flasks and incubated in a Dubnoff metabolic shaker at 37° for 10 min to allow the metabolism to reach equilibrium. To each flask was added 0·5 ml of a solution containing 16,600 dis./min (2·78 nmoles) of 8-^{14}C-mescaline-hydrochloride and the incubation continued for various times. At the end of incubation the contents of the flasks were subjected to differential centrifugation.

The procedure for the extraction of radioactivity is reported elsewhere.[5] Unchanged mescaline was separated from its metabolites[3] on columns 50 mm long with an internal diameter of 4·2–4·5 mm and containing Dowex 50W-X_4, 200–400 mesh previously buffered at pH 5·8 with phosphate buffer.[6] The overall recovery through the isolation procedure ranged from 72 to 78 per cent; the values reported are corrected for the recovery. The radioactivity was assayed in a liquid scintillation spectrometer in a manner similar to that previously reported.[5]

The animals exhibited gross behavioral changes characterized by agitation and excitement within 30 min after the administration of 50 mg/kg of mescaline. This behavior continued for 60–90 min with peak changes occurring at 60 min. The period of excitation was followed by a period of inactivity with weakness and uncoordinated movements of the hind legs. The animals treated with iproniazid or semicarbazide were slightly more active than the saline-treated controls. The animals pretreated with either iproniazid or semicarbazide followed by mescaline exhibited gross behavioral changes similar

* This work was presented in part at the 15th annual conference of the Association of Physiologists and Pharmacologists of India, held in Kanpur, U.P., December 27–29, 1969. The author gratefully acknowledges the financial support from the Indian Council of Medical Research, New Delhi. Sincere thanks are due to Dr. O. D. Gulati, Dean and Professor of Pharmacology, Medical College Baroda, India for the interest in the study.

to those observed in the animals treated with mescaline alone. The reserpinized animals exhibited signs characteristic of reserpine, i.e. diarrhoea, drowsiness with closure of the eyes and tranquillization. The administration of mescaline to reserpinized animals did not modify the reserpine-induced sedation.

The concentrations of unchanged 8-^{14}C-mescaline in the brain as a per cent of total radioactivity were: 87·0 per cent at 15 min; 86·3 per cent at 30 min; 81·0 per cent at 60 min; 80·0 per cent at 90 min; 61·5 per cent at 180 min. The concentration of mescaline reached a maximum in 60 min and then declined; at the end of 180 min, it had dropped to approximately 20 per cent of the peak level. The levels of mescaline in the brain of mice pretreated with iproniazid or reserpine were not significantly different from the control group.

The levels of unchanged labeled mescaline in the liver as a per cent of total radioactivity were: 78·2 per cent at 1 hr; 64·0 per cent at 2 hr; 53·5 per cent at 3 hr; 48·9 per cent at 4 hr. Pretreatment with reserpine or semicarbazide rendered very little effect on the hepatic levels. Pretreatment with iproniazid considerably elevated the formation of *N*-acetylmescaline; the level of unchanged mescaline remained unaffected at 1 hr but, compared with the control value, was significantly elevated at 3 hr.[6] Mescaline was found to be predominantly associated with the soluble-supernatant fraction and to a considerably lesser extent with the nuclear and particulate fractions obtained from brain and liver (Tables 1 and 2). When brain tissues from non-injected mice were homogenized in 0·25 M sucrose solution and incubated with 8-^{14}C-mescaline for various time intervals, a distribution pattern similar to that observed in intact animals resulted (Table 3).

From 16 to 22 per cent (320–440 mμc) of the administered ^{14}C was excreted in the urine within the first hr (Fig. 1). The amount of ^{14}C excreted at 3, 8 and 24 hr was 32–34 per cent (630–678 mμc), 38–40 per cent (765–810 mμc) and 61–67 per cent (1220–1350 mμc) respectively. Pretreatment with reserpine, iproniazid or semicarbazide had virtually no effect on the subcellular distribution of mescaline into the brain and liver and on the excretion of ^{14}C in the urine.

The functional significance of the bulk of unbound mescaline or LSD[4] in the whole brain or in the areas of brain is difficult to interpret but may have some bearing on the interaction of psychotogenic agents with brain biogenic amines as suggested by others.[7–9]

Inhibition of the amine oxidase pathway failed to alter the localization of mescaline into various subcellular fractions of brain or liver. Also reserpine exerted no influence on the distribution pattern of mescaline into various subcellular fractions of both brain and liver. This would imply that the injected mescaline perhaps does not occupy the amine-containing granules that were previously emptied of their contents by reserpine pretreatment.

TABLE 1. SUBCELLULAR DISTRIBUTION OF 8-^{14}C-MESCALINE IN BRAIN OF CONTROL MICE AND MICE PRETREATED WITH RESERPINE OR IPRONIAZID*

Fraction	15 min	30 min	60 min	90 min	180 min
			Control		
Nuclear	63 ± 5	147 ± 36	366 ± 41	271 ± 27	56 ± 8
Particulate	81 ± 11	113 ± 16	108 ± 26	96 ± 14	49 ± 6
Supernatant	876 ± 92	1228 ± 84	1417 ± 80	901 ± 71	283 ± 110
			Reserpine-pretreated		
Nuclear	186 ± 43	217 ± 30	232 ± 51	146 ± 34	123 ± 28
Particulate	85 ± 14	82 ± 21	69 ± 13	81 ± 6	60 ± 8
Supernatant	807 ± 168	1238 ± 65	1527 ± 111	966 ± 51	357 ± 66
			Iproniazid-pretreated		
Nuclear	65 ± 12	144 ± 24	339 ± 54	187 ± 22	84 ± 6
Particulate	141 ± 23	127 ± 20	254 ± 41	270 ± 34	89 ± 11
Supernatant	913 ± 104	1115 ± 64	1358 ± 143	972 ± 91	444 ± 38

* Values are expressed as pmoles of unchanged 8-^{14}C-mescaline/g of fresh tissue. The results are averages of five to six separate experiments ± standard deviation. The time intervals indicate the time of sacrifice after injection of 8-^{14}C-mescaline (2 μc).

TABLE 2. SUBCELLULAR DISTRIBUTION OF 8-^{14}C-MESCALINE IN LIVER OF CONTROL MICE AND MICE PRETREATED WITH RESERPINE, IPRONIAZID OR SEMICARBAZIDE*

Fraction	Control				Reserpine-pretreated (1 hr)	Iproniazid-pretreated (1 hr)	Semicarbazide-pretreated (1 hr)
	1 hr	2 hr	3 hr	4 hr			
Nuclear	1306 ± 126	563 ± 61	203 ± 48	215 ± 54	1466 ± 188	2323 ± 268	1430 ± 192
Particulate	1541 ± 158	631 ± 72	188 ± 36	236 ± 29	959 ± 97	1354 ± 125	828 ± 169
Supernatant	8094 ± 708	2638 ± 402	804 ± 139	521 ± 84	7543 ± 1055	9757 ± 1213	9295 ± 803

* Values are expressed as pmoles of unchanged 8-^{14}C-mescaline/g of fresh tissue. The results are averages of four to six separate experiments ± standard deviation. The time intervals indicate the time of sacrifice after injection of 8-^{14}C-mescaline (2 μc).

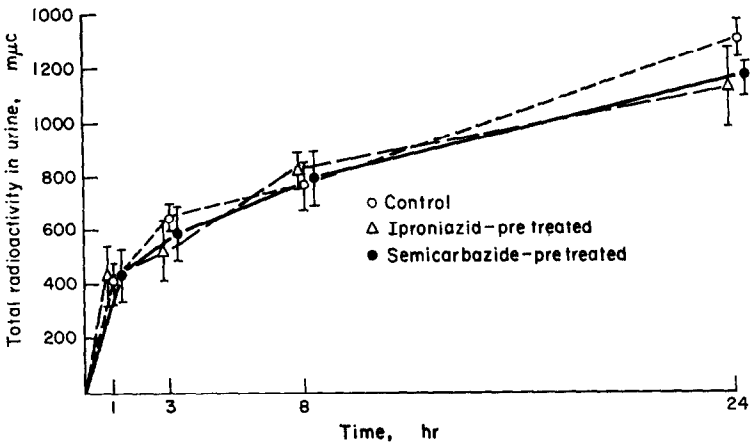

FIG. 1. Urinary excretion of radioactivity at various times following 2 μc of 8-^{14}C-mescaline i.p. The urine was collected at 1, 3, 8 and 24 hr. The results are averages of at least four separate experiments. The vertical lines designate the standard deviation. The figures on the ordinate represent the total radioactivity in the urine.

TABLE 3. SUBCELLULAR DISTRIBUTION OF 8-^{14}C-MESCALINE IN BRAIN HOMOGENATE: STUDIES *IN VITRO**

Fraction	15 min	30 min	60 min	90 min	180 min
Nuclear	949 ± 101	828 ± 76	1267 ± 112	1387 ± 128	972 ± 156
Particulate	525 ± 68	283 ± 69	444 ± 61	837 ± 115	615 ± 67
Supernatant	11,359 ± 851	12,510 ± 683	11,768 ± 1131	11,356 ± 933	11,531 ± 1438

* Brain homogenates from control mice were incubated at 37° with 8-^{14}C-mescaline for various time intervals. Values are expressed as pmoles of unchanged 8-^{14}C-mescaline/g of fresh tissue. The results are averages of more than four separate experiments ± standard deviation.

The amount of ^{14}C excreted by the kidney during the 24 hr following the injection of mescaline ranged from 1220 to 1350 mμc. Approximately 40–45 per cent of this amount was excreted in the first 3 hr. This relatively rapid elimination of mescaline appears to be due to poor binding of the psychotomimetic amine to various tissues.

Department of Pharmacology,
Medical College,
Baroda, India

NANDKUMAR S. SHAH*

REFERENCES

1. J. COCHIN, L. A. WOODS and M. H. SEEVERS, *J. Pharmac. exp. Ther.* **101,** 205 (1951).
2. N. NEFF, G. V. ROSSI, G. D. CHASE and J. L. RABINOWITZ, *J. Pharmac. exp. Ther.* **144,** 1 (1964).
3. K. D. CHARALAMPOUS, K. E. WALKER and J. KINROSS-WRIGHT, *Psychopharmacologia* **9,** 48 (1966).
4. S. H. SNYDER and M. REIVICH, *Nature, Lond.* **209,** 1093 (1966).
5. N. S. SHAH, A. KAMANO, S. GLISSON and D. CALLISON, *Int. J. Neuropharmac.* **7,** 75 (1968).
6. N. S. SHAH and H. E. HIMWICH, *Neuropharmacology* **10,** 547 (1971).
7. N. J. GIARMAN and D. K. FREEDMAN, *Pharmac. Rev.* **17,** 1 (1965).
8. A. BARBEAU, P. SINGH and M. JOUBERT, *Life Sci.* **5,** 757 (1966).
9. J. R. SMYTHIES, R. J. BRADLY and V. S. JOHNSTON, *Psychopharmacologia* **10,** 379 (1967).

* Present address: William S. Hall Psychiatric Institute, Columbia, S.C., U.S.A.