Short Communication

Pharmacology *15:* 283–287 (1977)

Binding of Mescaline with Subcellular Fractions upon Incubation of Brain Cortex Slices with [^{14}C] Mescaline

R.K. Datta, W. Antopol † and J.J. Ghosh

Departments of Pathology, Beth Israel Medical Center; Mount Sinai School of Medicine of The City University of New York, New York, N.Y., and Department of Biochemistry, University College of Science, Calcutta University, Calcutta

Key Words. Binding of mescaline · Brain microsomes, mitochondria, nuclei, ribosomes, slices, soluble supernatant · Mescaline · Permeation of mescaline

Abstract. Incubation of brain cortex slices in the presence of glucose resulted in the permeation of about 65 % of [^{14}C] mescaline into slices. Of this, about one-third radioactivity was bound with nuclei, mitochondria, microsomes, and ribosomes. Dialysis of subcellular fractions did not markedly reduce the amounts of radioactivity bound to the fractions. The permeation into slices and the binding of mescaline to subcellular fractions were fairly time-dependent, but were inhibited by the presence of potassium cyanide, or by the absence of glucose and by heating to 80 °C for 1 min.

Mescaline sulfate decreases the protein synthesizing capacity of brain cortex slices when measured *in vitro* (*Datta and Ghosh,* 1971). Ribosomes of the mescaline-treated slices have decreased capacity to bind aminoacyl-tRNA (*Datta et al.,* 1974). In an attempt to find the mechanism involved in the decreased ribosomal binding of aminoacyl-tRNA, attention has been directed to study the binding of ribosomes with mescaline. In this attempt, the binding of other subcellular fractions with mescaline has also been studied. Brain cortex slices were incubated in the presence of [^{14}C] mescaline, washed and fractionated into nuclei, mitochondria, microsmes, ribosomes and soluble supernatant. Radioactivity in these fractions was measured. Results presented here indicate that these fractions bind mescaline, though ribosomes are least active in binding and that the binding process is energy- and time-dependent.

Received: May 25, 1976.

Table I. Subcellular localization of [^{14}C] mescaline upon incubation of brain cortex slices[1]

Fraction	Total cpm in subcellular fractions upon incubation of slices in the presence of				
	no glucose for 60 min[2]	glucose (0.2 *M*) for			KCN (10 m*M*) for 60 min[2]
		15 min[2]	30 min[2]	60 min[3]	
Washed slices	652	1,556	3,357	6,545 ± 607	601
Nuclei	93	220	430	890 ± 75	78
Mitochondria	56	123	256	495 ± 32	40
Microsomes	50	150	318	620 ± 55	60
Ribosomes	20	20	30	70 ± 10	22
Soluble supernatant	403	990	2,005	3,815 ± 340	329

[1] Brain cortex slices, 2.5 g wet weight, were suspended in 10 ml of Krebs-Ringer buffer, pH 7.0 in the presence of substances indicated above and incubated with [^{14}C] mescaline hydrochloride (10,000 cpm) at 37 °C for time intervals as indicated. Other details were as described in the 'Methods' section. Subcellular fractions were solubilized overnight in hyamine hydroxide and radioactivity measured in a liquid scintillation counter. All determinations were carried out in duplicate and results were accepted when duplicates did not differ by more than 5 %.

[2] Mean values of two experiments.

[3] Mean values ± SE of four experiments.

Methods

The cortex portions of brain tissue obtained from freshly slaughtered goats were carefully removed and cut into 0.5-mm slices in the cold. 2.5 g wet weight of cortex slices were suspended in 10 ml Krebs-Ringer buffer (0.15 *M* NaCl, 3 m*M* KCl, 2 m*M* $CaCl_2$ and 0.2 *M* $NaHCO_3$), pH 7.0, previously saturated with O_2 + CO_2 (95:5) and containing substances indicated in table I and [^{14}C] mescaline hydrochloride (mescaline-8-[^{14}C] hydrochloride), specific activity 5 mCi/m*M* (New England Nuclear Corporation). The respiring conditions of the slices were periodically checked by separate manometric procedure under similar conditions. After treatment at 37 °C for time intervals as indicated in table I, slices were collected by centrifugation and washed 5 times with 10-ml portions of Krebs-Ringer buffer to wash off residual radioactivity (as checked by its almost absence in the 5th wash). Slices were homogenized and fractionated into subcellular fractions. Nuclei were isolated by the method of *Hogeboom et al.* (1952) and washed 3 times with the buffer. The postnuclear supernatant was centrifuged at 12,000 *g* for 15 min to sediment the mitochondrial fraction which was washed 3 times with the buffer. The postmitochondrial supernatant was centrifuged at 60,000 *g* for 2 h in a Spinco Ultracentrifuge to sediment the microsomal fraction and to collect the soluble supernatant. The microsomal pellets were washed twice with the

buffer. Ribosomes were prepared as described by *Datta and Ghosh* (1963, 1970). Recovery of subcellular fractions was about 85 % on the basis of protein in the slices. Mescaline was identified by descending chromatography as described by *Daly et al.* (1962).

Results

Incubation of brain cortex slices in the presence of glucose for 1 h resulted in the permeation of a major portion (about 65 %) of [^{14}C] mescaline. Of this, about one-third bound with nuclei, mitochondria, microsomes and ribosomes, the rest remaining in the soluble supernatant (table I). In the presence of glucose, nuclei, mitochondria and microsomes bound fair amounts of mescaline, and ribosomes bound the least amount. The incubation of brain cortex slices in the absence of glucose decreased the permeation into slices and the binding of mescaline with subcellular fractions by about 90 %. So did the incubation in the presence of potassium cyanide (table I). The permeation into slices and the binding of mescaline with subcellular fractions in the presence of glucose was fairly time-dependent (table I). It was found that the preheating of the slices in a water bath at 80 °C for 1 min decreased drastically the permeation into slices and the binding of mescaline with subcellular fractions (table II). Dialysis of subcellular fractions in 50 vol of Krebs-Ringer buffer at 4 °C for 18 h did not markedly reduce the amounts of radioactivity bound to subcellular fractions (table II). The binding of mescaline to subcellular fractions was strong enough to withstand up to four washings with Krebs-Ringer buffer, pH 7.0. However, the treatment with sodium acetate buffer (0.1 *M*), below pH 5.0, 5 % trichloroacetic acid or 50 % ethanol disrupted the binding and washed out radioactivity significantly (table II). The radioactivity washed out by 50 % ethanol from nuclei, mitochondria and microsomes contained mescaline identifiable by descending chromatography.

Discussion

Even after the washing of brain cortex slices up to 5 times with buffer following incubation with [^{14}C] mescaline and the washing of subcellular fractions 2–3 times with buffer, radioactive mescaline was detected in the slices and fractions. In view of failure of these extensive manipulations to dislodge [^{14}C] mescaline from slices and subcellular fractions, it is assumed that the binding of mescaline with subcellular fractions is fairly strong. The washing out of mescaline from these fractions by 50 % ethanol suggests that mescaline is probably bound to lipid materials of the fractions. Since the permeation of and the binding of mescaline were very low in the absence of glucose or in the presence

Table II. Effects of various manipulations on the binding of mescaline with subcellular fractions[1]

Fraction	Total cpm in subcellular fractions upon manipulation of								
	no heating	heating[2]	no dialysis	dialysis[3]	no washing	washing with K-R buffer[4]	washing with acetate buffer[5]	washing with TCA[6]	washing with ethanol[7]
Washed slices	6,545	735	–	–	–	–	–	–	–
Nuclei	900	75	895	870	895	865	400	375	195
Mitochondria	502	47	500	482	525	507	275	203	112
Microsomes	626	69	610	601	615	595	295	312	192
Ribosomes	73	13	67	65	70	65	30	27	14
Soluble supernatant	3,823	480	–	–	–	–	–	–	–

[1] Brain cortex slices, 2.5 g wet weight, were suspended in 10 ml of Krebs-Ringer buffer, pH 7.0 in the presence of 0.2 *M* glucose and incubated with [^{14}C]-mescaline hydrochloride at 37 °C for 1 h. Subcellular fractions were isolated and manipulations as described above were carried out with them. Other details were as described in the 'Methods' section and in table I. Mean values of two experiments are tabulated.
[2] Slices were heated at 80 °C for 1 min and the subcellular fractions were isolated therefrom.
[3] In 50 vol of Krebs-Ringer buffer, pH 7.0 at 4 °C for 18 h.
[4] Four washings, 10 min each, with Krebs-Ringer buffer, ph 7.0 at 25 °C.
[5] One washing with 0.1 *M* sodium acetate buffer, pH 4.6 for 10 min at 25 °C.
[6] One washing with 5 % trichloracetic acid for 10 min at 25 °C.
[7] One washing with 50 % ethanol for 10 min at 25 °C.

of potassium cyanide, it is apparent that the permeation of mescaline into slices and the binding of mescaline with subcellular fractions are dependent on respiring conditions of brain cortex slices. As a major portion of mescaline permeated into the respiring brain cortex slices, the pharmacological action of mescaline is likely to be mediated through actively metabolizing brain tissue. It may also be likely that subcellular fractions which substantially bind mescaline may be involved, in some manners, in the mechanism of action of mescaline. Since subcellular localization of mescaline indicates that of all subcellular fractions, ribosomes are the least active in binding mescaline, the binding of mescaline with ribosomes may not be greatly responsible for the observed decreased capacity of their binding of aminoacyl-tRNA. Demethylation of mescaline occurs in brain cortex slices, and ribosomes are appreciably methylated by a transmethylating enzyme present in the soluble supernatant (unpubl. results). It may be likely that the methylation of ribosomes leads to the decrease of their aminoacyl-tRNA-binding capacity.

Acknowledgement

This study was supported by the Charles H. Silver Foundation, New York, Indian Council of Medical Research and the Council of Scientific and Industrial Research, New Delhi. The authors thank *Steven Friedfeld* and *Caryl Towner* for their assistance.

References

Daly, J.; Axelrod, J., and Witkop, B.: Methylation and demethylation in relation to the *in vitro* metabolism of mescaline. Ann. N.Y. Acad. Sci. *96:* 37–45 (1962).

Datta, R.K. and Ghosh, J.J.: Preparation and properties of brain cortex ribosomes. J. Neurochem. *10:* 363–372 (1963).

Datta, R.K. and Ghosh, J.J.: Mescaline-induced changes of brain cortex ribosomes. Effects of mescaline on the stability of brain cortex ribosomes. Biochem. J. *117:* 961–968 (1970).

Datta, R.K. and Ghosh, J.J.: Mescaline-induced changes of brain cortex ribosomes. Effects of mescaline on amino acid incorporating ability of ribosomes. Brain Res., Amsterdam *33:* 193–203 (1970).

Datta, R.K.; Ghosh, J.J., and Antopol, W.: Mescaline-induced changes of brain cortex ribosomes. Effect of mescaline on the binding of aminoacyl-tRNA to ribosomes of brain tissue. Biochem. Pharmac. *23:* 1687–1692 (1974).

Hogeboom, G.H.; Schneider, W.C., and Striebich, M.J.: Cytochemical studies. On the isolation and biochemical properties of liver nuclei. J. biol. Chem. *196:* 111–120 (1952).

R.K. Datta, Department of Pathology, Beth Israel Medical Center, *New York, NY 10003* (USA)