Naunyn-Schmiedeberg's Arch. Pharmacol. 296, 297–300 (1977)

Mescaline-Induced Changes of Brain-Cortex Ribosomes
Mescaline Demethylase Activity of Brain-Cortex Soluble Supernatant *

R. K. DATTA[1] and J. J. GHOSH[2]

[1] Departments of Pathology, Beth Israel Medical Center, New York, N.Y. 10003 and
Mount Sinai School of Medicine of The City University of New York, New York, N.Y. 10029, U.S.A.
[2] Department of Biochemistry of Calcutta University, Calcutta 700019, India

Summary. Brain-cortex slices demethylate mescaline and *p*-methoxyacetanilide, a reference *O*-demethylating substrate, though the rate of demethylation of mescaline is about one third that of the reference substrate. The demethylase activity is localized mostly in the soluble supernatant (105000 × g). It is purified 47-fold with respect to the demethylation of mescaline by ammonium sulfate precipitation and DEAE cellulose chromatography. The partially purified demethylase, which is stable for 3–5 days at −5° C in the presence of dithiothreitol and glutathione and is inhibited by *p*-chloromercuribenzoate, has maximal activity at pH between 7.2 and 8.0. It demethylates mescaline into 3,4-dimethoxy-5-hydroxyphenethylamine and 3,5-dimethoxy-4-hydroxyphenethylamine and some unidentified derivatives.

Key words: Brain-cortex slices – O-demethylase – Mescaline – Soluble supernatant.

INTRODUCTION

Mescaline sulfate decreases the protein-synthesizing ability of ribosomes of brain cortex slices when measured in vitro (Datta and Ghosh, 1971). Since mescaline is known to be demethylated in the liver (Daly et al., 1962), attention has been directed to examine whether there is a transmethylation process in the brain and if so, whether it is linked, in any way, to the decrease of protein-synthesizing ability of ribosomes as a result of mescaline treatment. In the present study, the enzymic demethylation of mescaline in the brain-cortex was examined. A demethylase activity was found in the soluble supernatant of brain-cortex slices. Its partial purification and its role in the demethylation of mescaline are presented here.

* 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, India. The authors thank Inderjit Kaur for her assistance in the preparation of the manuscript

METHODS

Preparation of Brain-Cortex Slices. The cortex portions of brain tissue obtained from freshly shaughtered goats were carefully removed and cut into 0.5 mm slices in the cold.

Preparation of Subcellular Fractions. Nuclei, mitochondria, microsomes, ribosomes and soluble supernatant were prepared as described by Datta and Ghosh (1963, 1970) and Datta et al. (1976).

Enzymic O-Demethylation of Mescaline. The *O*-demethylation of mescaline was studied in incubation mixtures as described by Axelrod (1956) by measuring formaldehyde formed by the method of Cochin and Axelrod (1959). Unless stated otherwise in the text or in tables or figures, the incubation (assay) mixture containing brain cortex slices or subcellular fractions or soluble supernatant or partially purified enzyme as demethylase source (with protein contents as specified in the text or in tables), 2 µmole of mescaline surface (or more as specified elsewhere) (or its congener p-methoxyacetanilide), 50 µmole of nicotinamide, 25 µmole of $MgCl_2$, 0.2 µmole of NADP, 0.1 mmole of sodium phosphate buffer, pH 7.4, 25 µmole of sodium isocitrate, 2.5 µg of isocitrate dehydrogenase, 5 µmole of neutralized semicarbazide hydrochloride (to trap formaldehyde) and water to make a final volume of 5 ml was placed in a 20 ml beaker. The mixture was incubated in a Dubnoff shaker for 1 h at 37° C in air. At the end of the incubation period, a 4 ml sample of the mixture was transferred to a 15 ml test tube containing 0.8 ml of 12 N HCl and assayed for formaldehyde. The enzyme activity was expressed as µmole of formaldehyde formed per h.

Identification of O-Demethylated Mescaline Derivatives. After completion of incubation as described above 1 ml of 12 N HCl was added to the whole incubate which was then kept in ice for 30 min. The precipitated protein was removed by centrifugation. The supernatant was lyophilized and the residue extracted with 5 ml of hot absolute ethanol. The extract was dried in vacuum. The residue was extracted 2 times with 2 ml portions of 0.1 N HCl to solubilize amines, i.e. the residual mescaline and its *O*-demethylated products. The combined extracts were evaporated to dryness in vacuum. The residue was extracted with 2.5 ml of absolute methanol and the extract was later concentrated in vacuum to 0.10–0.15 ml. The methanol extract (0.05 ml) was applied to the Whatman No. 1 filter paper.