SUB-CELLULAR DISTRIBUTION OF MONOAMINE OXIDASE AND MONOAMINE DEHYDROGENASE

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Summary: MAO and MADH activities in different sub-cellular fractions of rat brain have been studied. The sub-cellular distribution pattern of MAO and MADH was found to be same, mostly concentrated in the mitochondrial fraction, although activities were also present in microsome and nucleus.

Key words: rat brain, monoamine oxidation, monoamine oxidase, sub-cellular distribution, monoamine dehydrogenase

INTRODUCTION

Studies with amine-tetrazolium reductase system are currently gaining tremendous importance since it offers valuable information about the amine metabolism. The earlier work on enzymatic reduction of tetrazolium salts or other redox indicators in the presence of amines was extended by others (6), although it was not known whether the amine dehydrogenating system (MADH) was identical with monoamine oxidase (monoamine: O_2 Oxidoreductase (deaminating), E.C.1.4.3.4.) or not. However, recent studies on MADH indicate that these two enzymes are different (1-4). A preliminary account on the distribution pattern of monoamine oxidase (MAO) and monoamine dehydrogenase in different sub-cellular fractions of rat brain is described in this communication.

MATERIALS AND METHODS

Whole brain homogenates of adult albino rats were prepared as a 10 per cent suspension in 0.25 M sucrose.

Assay of MAO activity: The reaction mixture for MAO assay contained 0.02 M phosphate buffer pH 6.5, 0.0125 M semi-carbazide pH 6.5, 0.01 M tyramine and 50 *mg* of tissue homogenate in a final volume of 2 *ml*. The reaction mixture was incubated at 38°C for 30 min with a pre-incubation period of 5 min. Aldehyde formed was measured at 420 *mμ* in a Bausch & Lomb Colorimeter by the method as described by Guha and Ghosh (4) previously.

Assay of MADH activity: The reaction mixture for MADH assay consisted of 0.025 M phosphate buffer pH 7.0, 0.5 *mg* neotetrazolium chloride (NTC), 0.01 M tryptamine and 100 *mg* of tissue homogenate in a final volume of 2 *ml* NTC reduction was measured at 520 *mμ* in a Bausch & Lomb Colorimeter according to the method of Guha and Ghosh (4). Incubations were carried out at 38°C for 30 minutes with a pre-incubation period of 5 minutes.

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Preparation of sub-cellular fractions: The procedure was followed with some modifications as outlined by Koeming *et al* (5). The actual procedure was as given below:

Adult albino rat was decapitated and whole brain was rapidly removed, weighed and placed in nine volume of ice cold 0.32 M sucrose to obtain 10 per cent homogenate. The brain was homogenized in a Potter-Elvehjem homogenizer for 30 seconds. All subsequent preparations were carried out in ice-cold condition.

At first, the homogenate was spun at 800 g for 10 minutes in a low speed MSE Centrifuge. The resulting pallet was washed twice with 0.32 M sucrose and designated as the nuclear fraction (N). To obtain the mitochondrial fraction (M), the washings from the N-fraction and the original supernatant were combined and spun at 15,689 g for 20 minutes in a Sorvall Super-speed Model RC2-B Centrifuge. The resulting pallet was washed once with 0.32 M sucrose. The supernatant and washings from the M-fraction were combined and centrifuged in a Spinco Model L-preparative ultracentrifuge at 81,000 g for 95 minutes. The resulting microsomal pallet (P) was suspended without washing in 0.32 M sucrose and the supernatant from the P-fraction was designated as the soluble fraction. All the fractions were made as the 10 per cent suspension in 0.32 M sucrose.

RESULTS AND DISCUSSION

Table I shows the high content of MAO and MADH in the mitochondrial fraction and low content of both the enzymes in the microsomes. But the lowest activities are observed in nuclear fraction. On the other hand, the soluble supernatant portion shows no trace of activity for either MAO or MADH.

From the present results it becomes further clear that, MAO obtained from any sub-cellular fractions could oxidise tyramine but tyramine is not dehydrogenated by MADH except in the case of whole brain homogenate. Addition of NADP with tyramine failed to recover the activity of MADH. On the contrary, tryptamine alone is able to reduce tetrazolium salts. Hence, for estimation of MADH, tryptamine was used as sub-strate. It is also reported that another indoleamine, serotonin like tryptamine is also actively oxidised by MADH (2).

TABLE I: MAO and MADH activities in different sub-cellular fractions of rat brain.

Sl No.	*Fraction*	*Relative activity (per cent)*	
		MAO (E_{420})	*MADH* (E_{520})
1	Homogenate	100	100
2	Nucleus	15.5	22.5
3	Mitochondria	65.5	65
4	Microsome	27.5	30
5	Soluble supernatant	Nil	Nil

The above results also indicate that the distribution pattern of both the enzymes are more or less same. At the same time, it also indicates that MADH is different from MAO because MADH actively oxidises indoleamines.

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