Biochemical Pharmacology, Vol. 29, pp. 2049–2053

0006-2952/80/0715-2049 $02.00/0

MAO TYPES IN GUINEA PIG LIVER MITOCHONDRIA

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(*Received* 9 *August* 1979; *accepted* 6 *March* 1980)

Abstract—MAO of guinea pig liver mitochondria actively deaminated dopamine, tyramine, serotonin and 5-methoxy-tryptamine, while tryptamine, 5-methyl-tryptamine and 7-methyl-tryptamine were moderately deaminated. Very little deamination occurred when benzylamine, noradrenaline and β-phenylethylamine were used as substrates. The *in vitro* inhibition patterns of MAO of guinea pig liver mitochondria by some selective inhibitors were investigated in the presence of tyramine, tryptamine and serotonin. Tryptamine oxidation showed biphasic inhibition pattern with harmaline, clorgyline and Lilly 51641, while the inhibition curves in the presence of pargyline and deprenyl were sigmoidal. The inhibition curves for tyramine oxidation were biphasic with all the inhibitors except pargyline. Serotonin–MAO inhibition curves, on the other hand, were sigmoidal with all the inhibitors except Lilly 51641. Thermal treatment of guinea pig liver mitochondria produced rapid inactivation of serotonin and tryptamine oxidizing activity, while benzylamine oxidizing activity was found to be most thermostable.

Tissues rich in monoamine oxidase (MAO, EC 1.4.3.4) activity produce a pigment-like substance when incubated with indolealkylamines such as tryptamine, serotonin, etc. [1–4]. The production of pigment by guinea pig liver mitochondria in the presence of tryptamine was found to be inhibited by various MAO inhibitors [5], indicating the possible involvement of MAO in pigment formation. MAO appears to exist as type A and type B [6–9] and it is suggested that serotonin (5-HT) is a specific substrate for type A MAO, while tryptamine is metabolized by both types [7]. However, there are some conflicting reports with tryptamine which appears to be a substrate for type B of the enzyme from certain sources [10]. On the other hand, pig brain MAO, which is essentially a type B MAO, is nevertheless active towards 5-HT [11]. In order to get a clear picture of the role of MAO in pigment formation, it was deemed necessary to elucidate the nature of MAO types in guinea pig liver mitochondria and an attempt was made to characterize MAO types of guinea pig liver mitochondria employing type-specific substrates and some selective MAO inhibitors, the results of which are presented in this communication.

MATERIALS AND METHODS

The liver of male guinea pigs (500–600 g) were removed and placed immediately in ice-cold 0.25 M sucrose, blotted dry and homogenized in 0.25 M sucrose to give a 10% suspension (w/v) and the mitochondrial fraction was prepared by the method of Schneider and Hogeboom [12]. The mitochondrial fraction was washed twice with 0.25 M sucrose and finally resuspended in 0.25 M sucrose at a final concentration of 3.5–4.0 mg protein/ml and divided into suitable aliquots for storage at −5°. All experiments, unless stated otherwise, were performed with mitochondrial fractions which were stored for not more than two weeks after preparation and had been frozen and thawed only once. MAO activity remained unaltered during this storage period.

The standard reaction mixture for MAO assay system contained 0.025 M phosphate buffer, pH 7.5, 1.8–3.0 mg mitochondrial protein, 0.0125 M semicarbazide and 0.01 M amine substrate in a final volume of 2 ml. All incubations were performed at 37° with air as the gas phase. The enzyme activity was determined by measuring the aldehyde or ammonia formed by the methods of Green and Haughton [13] or Conway and Byrne [14], respectively. Preliminary experiments were performed to ensure that enzyme activity was linear with respect to time and enzyme concentration employed.

When MAO inhibitors (MAOI) were used, the enzyme was preincubated with the inhibitors at 37° for 30 min in the case of clorgyline, pargyline, deprenyl, Lilly 51641 and for 10 min in the case of harmaline. Among the different MAOI employed in the present study, harmaline has been reported by various workers to be a reversible inhibitor [15–17]. However, experiments to ascertain the nature of harmaline inhibition of guinea pig liver mitochondrial MAO by dialysis, preincubation of the enzyme with the inhibitor for varied times, Ackermann Potter plot [18] and washing the mitochondria several times following preincubation with the inhibitor according to Udenfriend *et al.* [15], rather suggested the irreversible nature of harmaline inhibition. To determine the thermal stability of the enzyme, mitochondrial suspension was kept at 55° for varying time intervals.

Protein concentration was determined by the method of Lowry *et al.* [19] using bovine serum albumin as the standard.

The substrate amines and harmaline were commercial products of Sigma Chemical Co., (St. Louis, MO, U.S.A.) and other reagents used in this study were of analytical grade.

RESULTS

The deamination by guinea pig liver mitochondrial preparation is maximum with dopamine as substrate, followed by tyramine, serotonin and 5-methoxy-tryptamine (Table 1). Tryptamine and its 5-methyl