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INHIBITION OF MONOAMINE OXIDATION IN RAT LIVER MITOCHONDRIA

P. S. BASU
Indian Institute of Experimental Medicine, Calcutta-32, India

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Abstract—The effects of various agents on inhibition of monoamine oxidase (MAO) and the amine dehydrogenating system (MADH) of rat liver mitochondria were studied. Cyanide and semicarbazide strongly inhibited MADH activity while MAO activity was not affected. MAO was found to be more susceptible to inhibition by tranylcypromine, pheniprazine, iproniazid and pargyline. The psychoactive agents tested inhibited MAO strongly whereas they inhibited MADH either feebly or not at all. Simultaneous addition of inhibitor and substrate did not show any appreciable inhibition of these enzyme systems by tranylcypromine, pheniprazine, iproniazid and pargyline. Degree of inhibition by these inhibitors was found to increase with increasing time of preincubation.

The earlier work on the monoamine oxidation by the enzymatic reduction of tetrazolium salts and other redox indicators [1–3] has been extended by others [4–6]. The histochemical studies using tetrazolium salt reduction in presence of monoamines have always been described in terms of monoamine oxidase (MAO) activity [7–12]. It is not known, however, whether the amine dehydrogenating system (MADH) is identical with MAO or not. Certain differences were observed [13] in the reactions catalysed by rat brain MAO and MADH systems by different MAO inhibitors. Partially purified MAO and MADH systems of rat liver mitochondria also behaved differently with respect to pH and substrate activity patterns [14]. The present study was, therefore, undertaken to determine whether rat liver MAO and MADH systems are also affected in a similar way by different inhibitors belonging to hydrazide and non-hydrazide types.

MATERIALS AND METHODS

Rat liver mitochondria prepared in isotonic sucrose according to the method of Schneider and Hogeboom [15] were suspended in 0·001 M phosphate buffer pH 7·0 and sonicated as 25 kc/s in an MSE magnetostrictor with 0·6% Triton X-100 for 3 min. The material was kept ice-cold during sonication and the suspension was then centrifuged at 144,000 *g*. for 1 hr in the Spinco Ultracentrifuge. The resulting clear supernatant was used, in the present study, as the source of MAO and MADH.

The reaction mixture for MADH assay consisted of 0·02 M phosphate buffer pH 7·5, 0·5 mg neotetrazolium chloride (NTC), 0·006 M tryptamine and 5 mg enzyme protein in a final volume of 2 ml. Reduction of NTC was measured at 540 nm by the method of Lagnado and Sourkes [5]. The reaction mixture for MAO assay contained 0·02 M phosphate buffer pH 7, 0·01 M tyramine, 0·0125 M semicarbazide hydrochloride neutralised to pH 7·0 and 5 mg enzyme protein in a final volume of 2 ml. MAO activity was measured at 420 nm according to the method of Green and Haughton [16] with slight modifications as described previously [17]. All the incubation mixtures were preincubated with or without inhibitors for 15 min prior to addition of the substrate and the incubations in presence or in absence of inhibitors were carried out at 37° for 30 min (maximum linearity) with air as the gas phase. Ammonia formed in these systems was determined by the manometric method of Braganca *et al.* [18]. Protein concentration was measured by the method of Lowry *et al.* [19] using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

The results presented in Table 1 indicate that MAO activity as expressed in terms of NH_3 formation was not affected by presence of KCN and semicarbazide. The production of diformazan however was very much reduced when KCN and semicarbazide were added to the complete MADH system though ammonia formation was found to be unaffected in the MADH reaction. It is evident from Table 1 that in the system without KCN, semicarbazide and NTC as well as in the system with KCN and semicarbazide, only MAO was operative but in the systems with only NTC or with KCN, semicarbazide and NTC, both MAO and MADH activities could be measured. It was found that none of the systems had effect on deamination of tryptamine by rat liver mitochondria, and uniform values of ammonia production was observed in all the systems. It is known that ammonia is one of the products of MAO reaction [20]. So if, theoretically, ammonia is also produced due to MADH reaction, production of ammonia in the system where both MAO and MADH are fully active should exceed that in the system in which only MAO is operative. But such an increase in ammonia formation did not occur in a system, where NTC reduction was not affected at all. This suggests that KCN and semicarbazide inhibits MADH reaction and that ammonia is not a product of MADH reaction, while MAO reaction in which KCN and semicarbazide are essential [21–23] is responsible for the production of ammonia. The possibility that the generation of the coloured material on the MADH assay could be due

Table 1. Tryptamine oxidation in rat liver mitochondria

Systems	MAO activity (μmoles NH_3/ 25 mg protein/ 30 min)	MADH activity (μmoles diformazan/ 25 mg protein/30 min)
Control*	51·0	—
Control + NTC	48·0	11·0
Control + KCN + semicarbazide	50·0	—
Control + KCN + semicarbazide + NTC	54·0	4·0

* Control system contained 25 mg protein, 0·05 M phosphate buffer (pH 7·0), 0·01 M tryptamine in a total volume of 2 ml.

0·5 mg NTC (neo-tetrazolium chloride), 0·01 M KCN and 0·04 M semicarbazide–HCl. (pH 7·0) were used in other experimental systems. The preincubation and the incubation times for all the systems were 15 min and 30 min respectively at 37°.

Table 2. Effect of various compounds on MADH and MAO activities of rat liver mitochondria

Inhibitors	Final concn. (M)	Per cent inhibition	
		MADH	MAO
Tranylcypromine	8×10^{-6}	65	100
Pheniprazine	10×10^{-6}	30	65
Iproniazid	1×10^{-6}	5	46
Pargyline	5×10^{-6}	7	66
Ephedrine	1×10^{-2}	Nil	57
Mescaline	1×10^{-3}	Nil	37
Rotenone	1×10^{-5}	Nil	61
Amytal	1×10^{-3}	Nil	31
Antimycin A	3×10^{-6}	Nil	41
Na-azide	1×10^{-3}	Nil	31
$\alpha\alpha'$-dipyridyl	1×10^{-3}	22	9
Cupric sulphate	1×10^{-3}	86	55

The systems were first incubated with or without inhibitors for 15 min at 37° followed by the addition of 0·006 M tryptamine or 0·01 M tyramine in MADH and MAO systems respectively. Diformazan or aldehyde formed were measured after further incubation for 30 min at 37°.

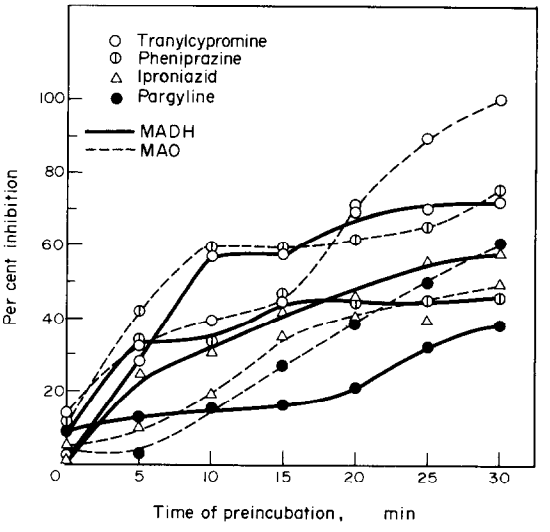

Fig. 1. Effect of variation of time of preincubation on inhibition of MADH of MAO by various inhibitors of monoamine oxidase. For MADH system the final inhibitor concentrations were: tranylcypromine 4×10^{-6} M; pheniprazine 5×10^{-6} M; iproniazid 5×10^{-6} M; Pargyline 1×10^{-5} M; and for MAO system final inhibitor concentrations were: tranylcypromine 2×10^{-6} M; pheniprazine 3×10^{-6} M; iproniazid 5×10^{-6} M; pargyline 1×10^{-7} M.

to a reaction between the aldehyde produced and NTC [9] has been ruled out by the observation that the amount of aldehyde formed by such systems is unaffected by the presence of NTC [13] and thus the effects of cyanide and semicarbazide on MADH activity cannot be due to competition with NTC for the aldehyde product.

It was further observed (Table 2) that MAO is more susceptible than MADH to inhibition by tranylcypromine, pheniprazine, iproniazid and pargyline. It was found that ephedrine, mescaline and rotenone failed to inhibit MADH activity. Amytal, antimycine-A, and sodium azide inhibited MAO activity while MADH activity was not affected at all. On the other hand, $\alpha\alpha'$-dipyridyl and cupric sulphate were more sensitive to inhibition of MADH activity than that of MAO reaction.

The results presented in Fig. 1 indicate that with both the enzyme systems, preincubation with the inhibitors is necessary for any significant degree of inhibition. The inhibitors do not have any appreciable influence when added simultaneously with the substrates. It was further observed that inhibition of MAO and MADH activities produced by MAO inhibitors could not be reversed by dialysis.

The above findings reveal certain similarities in the inhibition of MADH and MAO by various agents along with some dissimilarities, although the possibility of the dehydrogenase being a primary or secondary reaction step in the overall reaction catalysed by MAO was suggested [24], and responses to inhibitors like cyanide, semicarbazide, ephedrine, mescaline etc. of both the systems strongly favours the concept that the oxidase and the dehydrogenase are different enzyme systems.

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