TRANSFORMATION OF MITOCHONDRIAL MONOAMINE OXIDASES OF TYPES A AND B

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The selective inhibitor of type A monoamine oxidase (MAO) chlorglyline (unlike deprenil, an inhibitor of type B MAO) prevented the appearance of ability to deaminate histamine or AMP, qualitatively new properties for this object, in fragments of mitochondrial membranes from rat liver when incubated under aerobic conditions. The qualitative transformation of the catalytic properties under the influence of oxidizing agents is evidently undergone by type A but not by type B MAO.

KEY WORDS: monoamine oxidases of types A and B; transformation of monoamine oxidases; chlorgyline; deprenil; histamine; adenylic acid.

Purified preparations of monoamine oxidase (MAO) and membrane-bound MAO undergo transformation (qualitative alteration) of their catalytic activity under conditions leading to partial oxidation of SH groups [1]. As a result of this transformation the MAO acquires the ability to deaminate not only monoamines, but also other nitrogenous bases (histamine, nucleotides, for example) that do not belong to the list of MAO substrates.

The object of this investigation was to identify the type of MAO undergoing transformation in fragments of liver mitochondrial membranes.

At least two types of MAO are distinguished [5]: A and B. By definition, amine oxidases highly sensitive to the inhibitory action of chlorgyline [N-(2,4-dichlorophenoxy)propyl-N-methyl-2-propinylamine hydrochloride] belong to the group of type A MAO [2]; one of their characteristic substrates is serotonin (5-HT) [5]. The term type B MAO is applied to amine oxidases characterized by high sensitivity to the inhibitory action of deprinil (N-1-phenylisopropyl-N-methyl-2-propinylamine hydrochloride) [3]; among the biogenic amines, type B MAO specifically oxidizes β-phenylethylamine [5]. If the property of undergoing transformation of catalytic activity is a feature of only one of these types of MAO, the prevention of transformation of MAO by low concentration of the corresponding selective inhibitor of that particular type of MAO could be expected, for the presence of a functionally intact catalytic center is one of the conditions for the possibility of MAO transformation [1].

EXPERIMENTAL METHOD

The methods of isolating mitochondrial membrane from rat liver homogenate and of measuring MAO activity from the liberation of ammonia, sources of the chemical compounds used, and their characteristics were given previously in [6].

EXPERIMENTAL RESULTS

Preincubation of the mitochondrial fraction of rat liver homogenate with chlorgyline inhibited the deamination of 5-HT (a substrate of type A MAO) on the average by 80%, whereas deamination of β-phenylethylamine (a substrate of type B MAO) was inhibited by only 20% (Fig. 1). Preincubation of the mitochondria with depre-

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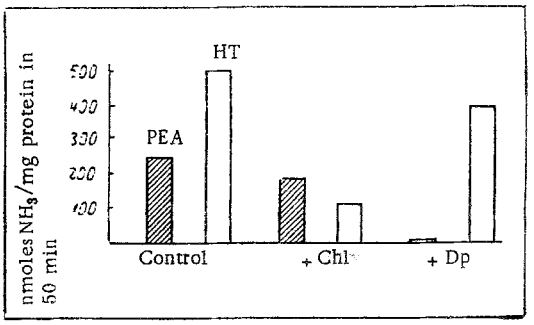

Fig. 1. Inhibition of deamination of β-phenylethylamine (PEA) and 5-HT in fraction of mitochondrial membrane of rat liver by chlorgyline (Chl) and deprenil (Dp). Suspensions (5 g of protein to 1 ml) of fragments of mitochondrial membranes in 0.01 M phosphate buffer (pH 7.4) preincubated for 60 min at 20°C either without addition of MAO inhibitors (control) or with Chl or Dp (final concentrations $5 \cdot 10^{-7}$ or 10^{-6} M, respectively), dialyzed against 600 vol of the same buffer for 48 h, after which deamination of PEA and 5-HT (final concentrations in samples $8 \cdot 10^{-4}$ and $6 \cdot 10^{-3}$ M, respectively) was investigated for 50 min at 37°C in an atmosphere of oxygen. Example of experiment (mean values calculated from results of four parallel determinations) from a series of three or four analogous experiments.

TABLE 1. Deamination of Nitrogenous Bases during Incubation with Fractions of Mitochondrial Membranes from Rat Liver

Treatment of mitochondria	Deamination, nmoles of ammonia/mg protein in 50 min			
	serotonin	β-phenylethylamine	histamine	AMP
Control	600	197	0	0
Preincubation with Cu^{2+}	165	77	143	43
Preincubation with chlorgyline	115	157	0	0
Preincubation with chlorgyline and then with Cu^{2+}	85	0	0	0
Preincubation with deprenil	480	13	23	0
Preincubation with deprenil and then with Cu^{2+}	135	0	110	23

Legend. Preincubation with Cu^{2+} (with 1 mM $CuSO_4$) [6] carried out at 4°C for 96 h; conditions of preincubation with chlorgyline or deprenil given in caption to Fig. 1. Control preincubated for 96 h at 4°C and then for 60 min at 20°C without Cu^{2+} and without MAO inhibitors. Final concentrations of histamine and AMP in samples 10 mM. Example of series including three analogous experiments shown.

nil inhibited the deamination of 5-HT by only 20% but completely blocked the activity of the type B MAO (Fig. 1), in agreement with data in the literature [3, 5].

It was found previously [6] that in the presence of Cu^{2+} cations as catalyst the oxygen of the air, partly oxidizing the SH groups of membrane-bound MAO, initiates the qualitative change (transformation) of their catalytic activity, as shown by the appearance of ability to deaminate various nitrogenous bases (histamine and

adenylic acid in the present experiments; see Table 1). Preliminary treatment with chlorgyline under conditions when this inhibitor largely blocked type A MAO activity (to judge from the deamination of 5-HT), but left type B MAO activity almost unchanged (tested by deamination of β-phenylethylamine), prevented the appearance of ability to deaminate histamine or AMP under these experimental conditions (Table 1). Conversely, deprenil, a type B MAO inhibitor, did not prevent the appearance of ability to catalyze these reactions in mitochondria incubated under aerobic conditions in the presence of Cu^{2+} cations. The results indicate that the ability to undergo transformation of catalytic activity under conditions favoring oxidation of SH groups is a feature of type A MAO but not of type B. It is known that MAO deaminates the most important of the neuromediators [5] and that transformation is particularly readily undergone by the MAO of brain tissue [4].

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INTERACTION BETWEEN 2,6-DIMETHYL-3,5-DICARBETHOXY-1,4-DIHYDROPYRIDINE AND ENZYMES OF THE NADPH-SPECIFIC ELECTRON TRANSPORT CHAIN OF RAT LIVER MICROSOMES

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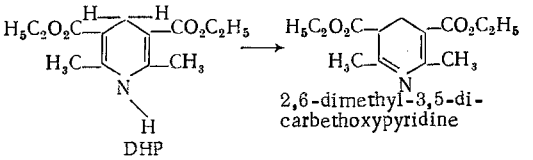
2,6-Dimethyl-3,5-dicarbethoxy-1,4-dihydropyridine interacts with the NADPH-dependent electron transport system of rat liver microsomes: It forms a type 1 complex with the terminal oxidase (cytochrome P-450) and also definitely inhibits the activity of NADPH-cytochrome c reductase and methindione demethylase. In experiments in vivo repeated administration of the compound had no inducing action on microsomal enzymes.

KEY WORDS: liver microsomes; electron transport system; 2,6-dimethyl-3,5-dicarbethoxy-1,4-dihydropyridine; antioxidants.

The compound 2,6-dimethyl-3,5-dicarbethoxy-1,4-dihydropyridine (DHP) possesses marked antioxidant and antiradical activity [1, 2, 6]. By oxidation of DHP in vivo 2,6-dimethyl-3,5-dicarbethoxypyridine is formed.



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