EFFECT OF ALCOHOLS ON MITOCHONDRIAL MONOAMINE OXIDASE ACTIVITY

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The effect of alcohol on mitochrondrial monoamine oxidase activity was studied by the use of rat liver mitochondria and highly purified preparations of the enzyme from rat and pig liver. The benzyl, β-phenylethyl, and octyl alcohols were found to inhibit the deamination of tyramine and 4-amino- and 4-methoxy-derivatives of β-phenylethylamine competitively and reversibly but to have practically no effect on the deamination of β-phenylethylamine and its 4-chloro-derivatives. The aliphatic alcohols exhibited inhibitory properties only if the length of the hydrocarbon chain was five methylene residues.

KEY WORDS: liver monoamine oxidase; action of alcohols; active center of enzymes.

Investigations conducted by the author for some years into the relationship between the chemical structure of amines and the activity of mitochondrial monoamine oxidase (MAO) [4] and also the selective inhibition of deamination of different amines by specific inhibitors [3] have led to the establishment of a hydrophobic region and a polar site in the active center of MAO responsible for binding amines [12].

Depending on their chemical structure, during interaction with MAO amines react with either one or two such sites. Depending on whether different inhibitors block one or both substrate-binding sites, deamination of individual amines is inhibited to a different degree [5]. Alcohols, closely similar to amines in their chemical structure, are particularly interesting in this respect. Octyl alcohol is known to inhibit MAO activity [14]. Data have been published on the effect of ethanol on biogenic amine metabolism [11, 13].

The effect of certain aliphatic-aromatic and aliphatic alcohols on the deamination of amines closely similar in their chemical structure – tyramine, β-phenylethylamine, and its 4-methoxy-, 4-amino-, and 4-chloro-derivatives – by rat liver mitochondria and also by highly purified preparations of MAO from rat and pig liver.

EXPERIMENTAL METHOD

Rat liver mitochondria were isolated from a 10% homogenate as described previously [4]. Highly purified preparations of MAO from rat liver (solubilized with the aid of detergent) [2] and from pig liver (solubilized without detergent) [9] were obtained by methods developed previously [2, 12]. Enzyme preparations used were purified by 50 times (rat liver) [2] and 380 times (pig liver) [12].

MAO activity was determined from the liberation of ammonia by Conway's isothermic distillation method followed by nesslerization [4]. The substrates were added in saturating concentrations, which for tyramine, the 4-amino-, 4-methoxy-, and 4-chloro-derivatives of β-phenylethylamine and for β-phenylethylamine itself were 8, 8, 6, 4, and 4 μmoles, respectively.

EXPERIMENTAL RESULTS AND DISCUSSION

The effect of some aliphatic-aromatic and aliphatic alcohols on the deamination of tyramine, β-phenylethylamine, and its 4-amino-, 4-methoxy-, and 4-chloro-derivatives by rat liver mitochondria and by

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TABLE 1. Effect of Alcohols on Deamination of β-Phenylethylamine and Its 4-Hydroxy-, 4-Amino-, 4-Methoxy-, and 4-Chloro-Derivatives by Mitochondrial MAO*

Inhibitor	Concn. of inhibitor (mM)	Substrate (derivative of β-phenylethylamine)	Inhibition (%)		
			A	B	C
Ethyl alcohol	1	4—OH	0	1	0
		4—NH_2	2	3	
		4—Cl	0	1	
		4—H	0	0	1
Butyl alcohol	1	4—OH	2	3	0
		4—NH_2	3	2	
		4—Cl	3	0	
		4—H	4	0	1
Amyl alcohol	1	4—OH	27	21	25
		4—NH_2	21	20	
		4—Cl	0	0	
		4—H	2	4	0
Isoamyl alcohol	1	4—OH	23		
		4—NH_2	21		
		4—Cl	0		
		4—H	0		
Octyl alcohol	1	4—OH	46	45	55
		4—NH_2	45	46	
		4—Cl	9	5	
		4—H	2	3	2
Benzyl alcohol	1	4—OH	42	42	50
		4—NH_2	43	43	
		4—Cl	11	2	
		4—H	0	2	6
	10	4—OH	65		
		4—H	40		
		BA†	52		
β-Phenylethyl alcohol	1	4—OH	37		
		4—OCH_3	38		
		4—NH_2	60		
		4—Cl	15		
		4—H	3		
β-Cyclohexyl alcohol	1	4—OH	39		
		4—OCH_3	45		
		4—NH_2	60		
		4—Cl	17		
		4—H	4		

* Values of inhibition (in % of breakdown of substrates without inhibitors) are given.
† Benzylamine, 10 μM.
Legend: A) suspension of fresh rat liver mitochondria; B and C) purified preparations of MAO from rat and pig liver.

purified preparations of MAO from rat and pig liver is shown in Table 1. Clearly benzyl alcohol in a final concentration of 10 mM inhibited the deamination of tyramine, β-phenylethylamine, and benzylamine by rat liver mitochondria fairly strongly. However, with a decrease in the concentration of benzyl alcohol to 1 mM a marked selectivity was observed in the inhibition. The deamination of the 4-hydroxy-, 4-methoxy-, and 4-amino-derivatives of β-phenylethylamine was inhibited by a much greater degree than that of unsubstituted β-phenylethylamine and its 4-chloro-derivatives. β-Phenylethyl β-cyclohexyl, and octyl alcohols show an analogous inhibiting action.

The inhibition of MAO activity in the presence of the above alcohols was competitive and reversible, and was completely abolished as a result of dialysis against 0.1 M phosphate buffer, pH 7.3, not containing the inhibitor. The inhibitory properties of the alcohols appeared only in homologues with more than five methylene groups in the aliphatic chain. Ethyl and butyl alcohols in concentrations ten times higher than those indicated in Table 1 did not inhibit MAO activity. The inhibitory properties of amyl and isoamyl alcohol were similar, but not significant. Meanwhile octyl alcohol caused the same inhibition as benzyl and the other aliphatic-aromatic alcohols tested.

Evidence has been obtained [12] of the existence of substrate-binding sites in the active center of MAO and on this basis the writer considers that the principal role in the binding of unsubstituted β-phenylethylamine, its 4-chloro-derivative, and benzylamine is played by purely hydrophobic interaction. The 4-hydroxy-, 4-methoxy-, and 4-amino-derivatives of β-phenylethylamine are additionally bound with MAO by their functional groups with a particular polar site in the active center of the enzyme.

Inhibition of the deamination of tyramine, β-phenylethylamine, and benzylamine (Table 1) by relatively high concentrations of benzyl alcohol can be explained by the fact that the benzyl alcohol competes with substrates for the hydrophobic region in the active center of MAO. Meanwhile the selectivity of inhibition observed on reducing the concentration of the alcohols (to 1 mM) was evidently due to the ability of the hydroxyl group of the alcohols to react additionally with the polar site of the active center, playing an important role in the binding of tyramine and of the 4-methoxy- and 4-amino-derivatives of β-phenylethylamine.

This hydrophobic region has a certain fixed size. The inhibitory properties of the aliphatic alcohols thus begin to appear only with homologues containing at least five methylene residues in their chain.

The inability of ethyl alcohol in the concentrations used (1-10 mM) to inhibit MAO activity is in agreement with data in the literature. The very slight (20%) inhibition by ethanol of the deamination of tyramine by mouse liver homogenate has been shown [10] to be observed only if the concentration of ethanol is of the order of 30 mM. According to other observations [6], which have not yet been explained, ethyl alcohol in a final concentration of the order of 0.02 mM actually stimulates (by about 20%) the oxidation of tyramine if incubated with rat liver homogenate at 4°C. Under the same conditions, but using rat liver homogenate solubilized with detergent, ethanol did not change the deamination of tyramine. The results (Table 1) indicate that the general rules established by the experiment with alcohols for mitochondria apply completely if MAO preparation from rat and pig liver, solubilized and purified by different methods, are used.

These results thus confirm the view that the known effect of ethanol on the metabolism of biogenic amines is unconnected with the blocking of MAO activity [7, 8].

The identical nature of the results reflecting the effect of alcohols on the deamination of the amines used by rat liver mitochondria and by purified MAO preparations from rat and pig liver may point to an identical (for the livers of different animals) mechanism of blocking of MAO activity in the presence of alcohols.

LITERATURE CITED

1. I. S. Severina, Biokhimiya, 38, 608 (1973).
2. I. S. Severina and N. I. Zhivotova, Biokhimiya, 38, 202 (1973).
3. I. S. Severina and I. S. Kozlova, Biokhimiya, 38, 1076 (1973).
4. I. S. Severina and T. N. Sheremet'evskaya, Biokhimiya, 32, 843 (1967).
5. I. S. Severina and T. N. Sheremet'evskaya, Biokhimiya, 34, 125 (1969).
6. G. C. Cotzias and J. L. Greenough, Nature, 185, 384 (1960).
7. V. E. Devis and M. J. Walsh, Science, 167, 1005 (1970).
8. A. Feldstein, H. Hoagland, H. Freeman, et al., Life Sci., 6, 53 (1957).
9. G. Hollunger and L. Oreland, Arch. Biochem., 139, 320 (1970).
10. L. S. Maynard and V. J. Schenker, Nature, 196, 575 (1962).
11. G. Rosenfeld, Proc. Soc. Exp. Biol. (New York), 103, 144 (1960).
12. I. S. Severina, Europ. J. Biochem., 38, 239 (1973).
13. J. Towne, Nature, 201, 709 (1964).
14. E. A. Zeller, in: J. Cheymol and J. R. Boessier (editors), Monoamine Oxidase Inhibitors: Relationship between Pharmacological and Clinical Effects, Oxford (1968), p. 3.