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DIFFERENCES BETWEEN AMINE OXIDASES DEAMINATING MESCALINE AND THE STRUCTURALLY RELATED 3,4-DIMETHOXYPHENYLETHYL AMINE

Z. HUSZTI and J. BORSY

Research Institute of Pharmaceutical Chemistry, Department of Pharmacology, Budapest, Hungary

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Abstract—Experiments were carried out with the compound T-134 to discriminate enzymes deaminating the structurally related amines. T-134 acts as a competitive inhibitor on mescaline deamination, while it does not exert any action on the degradation of DM and tyramine. Experiments were made also with "mixed substrates and with octanol and semicarbazide" to confirm our results obtained with T-134. The enzymes degrading the two amines can be separated by the added oxygen consumptions of the mixed substrates by their resistance or sensitivity towards octanol and semicarbazide, respectively. The assumption—that DM is destroyed by MAO in contrast to mescaline—was supported by measurements on competition between DM and serotonin. It is postulated, that DM decreased the serotonin degradation by rat liver homogenate. The fact that different enzymes are responsible for the deamination of DM and mescaline may explain the pharmacological differences between amines.

THE EFFECTS and metabolism of 3,4-dimethoxyphenylethyl amine (DM) have come to the forefront of the interest recently. This compound was first isolated and identified from the urine of acute schizophrenics.[1,2] In normal urine no existence of DM has been found.[3] According to Friedhoff's assumption it results from the disturbed metabolism of dopamine in schizophrenia.[3] Experimental evidence of this suggestion was also furnished by Friedhoff and his co-workers[4] who could identify 3,4-dimethoxyphenyl acetic acid in the urine of schizophrenics after dopamine infusion. Probably this reaction is due to O-methyl-transferase properties which were studied in more detail by Axelrod.[5] Further oxidation of DM, producing the acid metabolite, is performed presumably by amine oxidase, according to the first *in-vitro* studies of Bhagvat and Blaschko.[6]

We have studied the *in-vitro* deamination of DM in relation to the interesting problem of mescaline metabolism.[1] We applied the method of 'inhibitor spectra' [7] to discriminate amine oxidases deaminating the structurally related amines.

MATERIALS AND METHODS

For enzyme activity measurements mescaline sulphate Merck, tyramine hydrochloride Fluka, serotonin, creatinin sulphate Fluka and 3,4-dimethoxyphenylethyl

Abbreviations used in the text:
DM = 3,4-dimethoxyphenylethyl amine.
MAO = monoamine oxidase.
DAO = diamine oxidase.

amine sulphate were used. The compound T-134, N(3,4,5-trimethoxy benzoyl)-N'-(betaoxy propyl) piperazine 3',4',5'-benzoic ester hydrochloride, was synthetized in our Institute by Toldy and Toth.[8] Semicarbazide hydrochloride (B.D.H. Ltd.) and octanol were analytical grade reagents.

Fractionation of rabbit liver tissues for the isolation of mitochondria by differential centrifugation in 0·25 M sucrose was performed according to the method of Burkard *et al.*[8] The mitochondria were taken up in 0·1 M phosphate buffer and the suspension was dialysed for 16 hr at 5° against distilled water before the lyophilization.[9] Nitrogen contents of the mitochondial preparations determined by the micro-Kjeldahl method amounted to 8–10 per cent.

Standard assays of amine oxidases activity were carried out in Warburg vessels at 38°. 20–60 mg of lyophilized mitochondrial preparation was suspended in 1 ml 0·1 M phosphate buffer, pH 7·4, and 5×10^{-3} to 5×10^{-2} M of inhibitor in 0·3 ml, was added to the enzyme. The mixture was made up to a total volume of 3 ml. The side arm contained buffered amine to yield a final concentration of $6{\cdot}6 \times 10^{-3}$ M. The inner well of the vessels held a strip of pleated filter soaked with 10% sodium hydroxide. Enzyme and reagent blanks were run in the same manner. MAO-activity measurements were carried out according to Creosey.[10] Q_{ox} and Q'_{ox} values were calculated as micro atoms of oxygen consumed per hr per mg protein or per g tissue respectively. For the determination of serotonin concentration we used the spectrophotometric method of Zile.[12] The reaction mixtures contained substrate and homogenate amounts similar to previously described.[13]

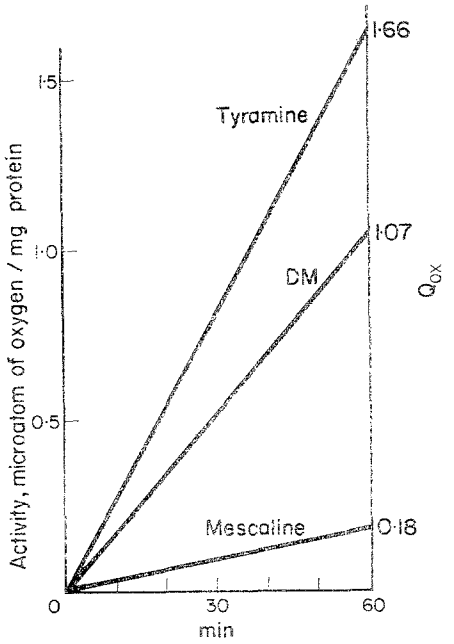
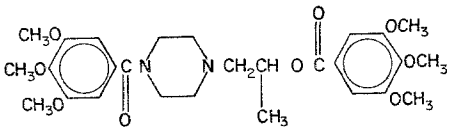


FIG. 1. Oxygen consumptions of rabbit liver mitochondria in the presence of tyramine, DM and mescaline. Standard assay was carried out with lyophilized rabbit liver mitochondria equivalent to 23·5 mg protein in a total volume of 3 ml per manometric vessel. Q_{ox} value for tyramine, DM and mescaline were 1·66, 1·07 and 0·18 respectively.

RESULTS

We have demonstrated the oxidation rates of tyramine, DM and mescaline, respectively by rabbit liver mitochondria (Fig. 1). Different velocities can be observed using the same mitochondrial preparation, but different amines as substrates. Specific activities of rabbit liver mitochondria, expressed in Q_{ox}, were 1·07 and 0·18 for DM and mescaline, respectively.

Our new compound T-134, representative of the piperazine derivatives (possessing a rather high anti-ulcerogen effect) was tested against mescaline oxidase of rabbit liver. The structure of this compound is presented in Fig. 2.



T 134

FIG. 2. Chemical structure of T-134.

Addition of 5×10^{-3} M and 10^{-3} M of T-134 to the incubation mixture gave a rather high effect on mescaline deamination, but no effect on the oxidation of DM and tyramine could be observed (Table 1).

TABLE 1. ACTION OF T-134 ON AMINE OXIDASES DEAMINATING MESCALINE, DM AND TYRAMINE

Substrate			Inhibitions (%)		
			T-134		
Name	Final conc. (M)	Control Q_{ox}	(5×10^{-3} M)	(10^{-3} M)	(5×10^{-4} M)
Mescaline	$6{\cdot}6 \times 10^{-3}$	0·17	85	65	40
DM	$6{\cdot}6 \times 10^{-3}$	1·07	0	0	0
Tyramine	$6{\cdot}6 \times 10^{-3}$	1·83	0	0	0

Standard assays were carried out as it was described in Methods. Each vessel contained rabbit liver mitochondria equivalent to 23·5 mg protein.

We plotted our results according to Lineweaver and Burk to obtain some information about the type of the inhibition (Fig. 3).

Data with octanol and semicarbazide are summarized in Table 2. No, or hardly any, action of semicarbazide, was observed. A rather high effect of octanol on the DM deamination could be observed.

Subsequent experiments were carried out with mixed substrates. Figure 4 shows the oxygen consumption in the presence of mescaline or DM and that of adding the two substrates simultaneously to the mixture.

Oxygen consumption in the presence of DM and serotonin were presented in Figure 5. Q'_{ox} values, calculated from the initial rate of the reaction were: 56 and 26 for serotonin and DM respectively.

When DM and serotonin were simultaneously added to the mixture, a rather pronounced effect of DM on the serotonin degradation can be observed. Kinetic studies carried out on the serotonin degradation in the presence of DM have shown that inhibition of serotonin oxidation may be considered as a "mixed type" one.

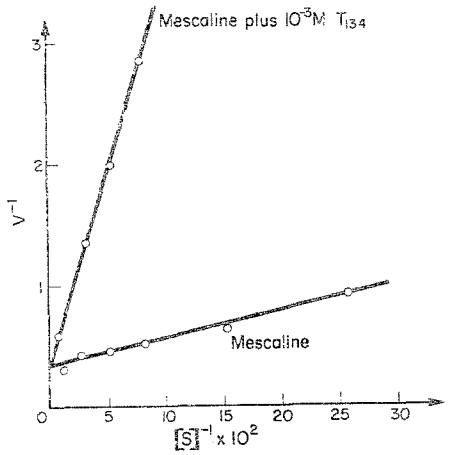


FIG. 3. Competitive inhibition of mescaline deamination by T-134. Abscissa: moles mescaline per liter; Ordinate: μl oxygen/hr/mg protein. The rate of oxidation was measured under standard conditions: phosphate buffer pH 7·4, oxygen atmosphere 38°, 80 mg lyophilized mitochondria equivalent to 62 mg protein.

TABLE 2. ACTION OF OCTANOL AND SEMICARBAZIDE ON AMINE OXIDASES DEAMINATING MESCALINE, DM, AND TYRAMINE

Substrate			Inhibitions %	
Name	Final conc. (M)	Control (Q_{ox})	Sat. Octanol	10^{-3} M Semi-carbazide
Mescaline	$6{\cdot}6 \times 10^{-3}$ M	0,18	0*	100*
DM	$6{\cdot}6 \times 10^{-3}$ M	1,17	90	20
Tyramine	$6{\cdot}6 \times 10^{-3}$ M	1,80	88	20

* = from Zeller *et al.*[14]

Standard assays were carried out as it was described in Methods. Each vessel contained rabbit liver mitochondria equivalent to 12–30 mg protein.

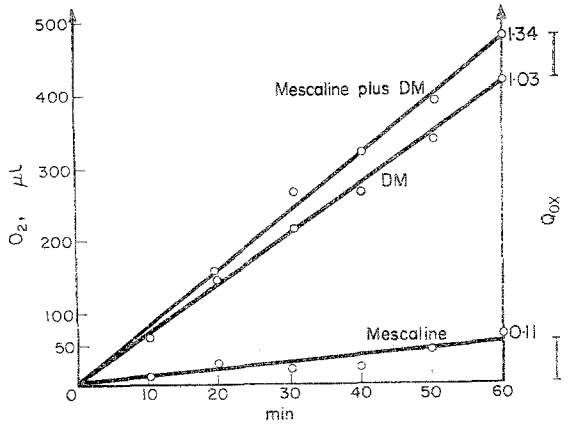


FIG. 4. Oxygen consumptions in the presence of DM and mescaline and in the presence of "mixed" substrates. Standard assays was carried out as it was described in Methods. Each vessel contained rabbit liver mitochondria equivalent to 33·2 mg protein. In the first case mescaline and DM were added alone to the incubation mixture however in the second case the substrates were mixed before addition.

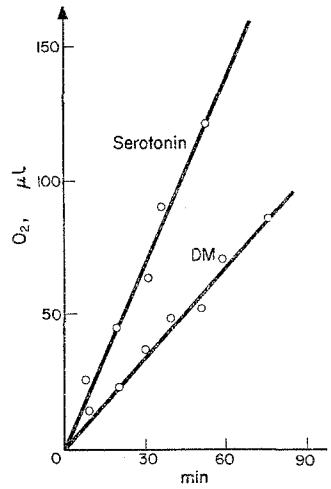


FIG. 5. Oxygen consumptions of rat liver homogenate in the presence of serotonin and DM respectively. Standard assay was carried out by Creasey's method. The side arm contained buffered serotonin or DM to yield a final substrate concentration of $6{\cdot}6 \times 10^{-3}$ M. The inner well of the vessel held a strip with 0·2M KCN to absorb the liberated ammonia. Incubation mixtures included 10^{-3} M cyanide and 10^{-3} M semicarbazide to block secondary oxidations. Each vessel contained rat liver homogenate equivalent to 220 mg of tissue.

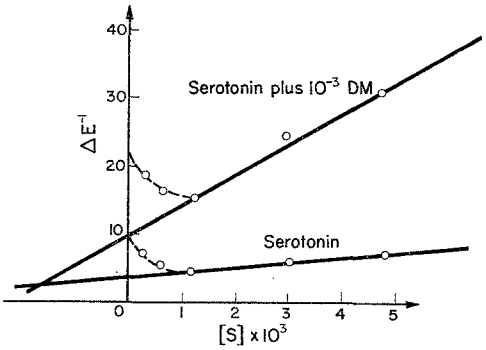


FIG. 6. Inhibition of serotonin deamination by DM. Abscissa: moles serotonin/l.; Ordinate: △ E/100 mg tissue (30 minutes). Standard assays were carried out by Zile's spectrophotometric method. The differences between serotonin concentrations measured before and after the reaction were expressed in extinction values.

DISCUSSION

Our experiments with T-134, octanol and semicarbazide suggest that different enzymes might participate in the respective deaminations of DM and mescaline. This assumption was confirmed by the competition reactions carried out with DM and mescaline, when a pronounced increase of the oxygen consumptions might be observed. Data obtained in the measurements of serotonin degradation by rat liver homogenate agree with our suggestion that DM is oxidized similarly to other monoamines by MAO. Previous assumptions that mescaline deamination is carried out by the so called "mescaline oxidase" belonging to the group of diamine oxidases was also confirmed,[14] but our data obtained with T-134 suggest a slight difference between

mescaline oxidase and soluble diamine oxidases. According to our previous results T-134 does not exert any action on soluble diamine oxidases.[15] That T-134 acts competitively on the mescaline deamination indicates that the alteration may exist in the active sites of enzymes. Our suggestion—that different enzymes are responsible for the deamination of DM and mescaline—may explain the differences between their pharmacological action reported by Smythies *et al.*[16] and recently by Borsy *et al.*[17]

According to Zeller's suggestion of steric factors having similar role in the interaction between chemoreceptors and certain drugs as in the formation of enzyme-substrate complexes, we may assume a parallelism between the pharmacological and biochemical action of these amines.[18]

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