Biochemical Pharmacology, Vol. 20, pp. 2571–2577. Pergamon Press, 1971. Printed in Great Britain

EFFECT OF MONOAMINE OXIDASE INHIBITORS ON QUALITATIVE ALTERATIONS IN ENZYMATIC PROPERTIES OF MITOCHONDRIAL MONOAMINE OXIDASES

MOHAMMED M. ABDEL SAMED, ZH. I. AKOPYAN, I. V. VERYOVKINA, A. A. KULYGINA and V. Z. GORKIN

Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the U.S.S.R., Moscow

(*Received* 14 *January* 1971; *accepted* 25 *March* 1971)

Abstract—Preincubation of highly purified ox liver MAO with specific MAOI pargyline, iproniazid or tranylcypromine prevents qualitative alteration ("transformation") in enzymatic properties of MAO after treatment of the enzyme with oxidized oleic acid (OOA). Pretreatment with pargyline or tranylcypromine of highly purified rat liver MAO prevents qualitative alteration in its enzymatic properties after incubation with Cu^{2+} in aerobic conditions. Pretreatment of rats with iproniazid prevents appearance in liver mitochondria of histamine-, putrescine- and L-lysine-deaminating activity after parenteral administration of OOA into the rats. It is possible that MAOI may find new fields of application based on their property to prevent qualitative alteration in enzymatic properties of monoamine oxidases in some pathological states (e.g. those accompanied by accumulation of lipid peroxides in tissues).

HIGH specific monoamine oxidase (MAO)(EC 1.4.3.4) activity is found in various membrane structures of liver cell.[1,2] But in mitochondrial membranes the amount of total MAO activity is far more than in other subcellular components.[3] In regulation of some functions of mitochondrial membranes higher unsaturated fatty acids (e.g. oleic acid) take part.[4] Sodium oleate inhibits deamination of tyramine by rat liver mitochondria.[5] Increase in concentration of peroxidation products in oleate increases the inhibitory effect on mitochondrial MAO activity.[6] This inhibitory effect is due to oxidation of SH groups[6] which are probably localized[7–9] out of the active site of MAO. In presence of saturating concentration of 5-hydroxytryptamine (5-HT) [or of α-methyltryptamine[11] and harmine[12]—selective competitive inhibitors of oxidative deamination of serotonin] oleic acid (OOA) causes not only a decrease in rates of deamination of monoamines but induces appearance[13–15] in mitochondrial membranes of a new ability (never exhibited by mitochondria in usual conditions) to catalyse deamination of histamine (substrate for diamine oxidase (DAO) of mammalian origin), lysine (substrate for DAO from some plants), and spermine (substrate for blood serum amine oxidase). This phenomenon, operationally termed "transformation" of MAO, has been observed also in experiments with highly purified MAO from ox liver mitochondria[16] or from a microorganism *Sarcina lutea*.[17] In experimental pathological states, accompanied by accumulation of lipid peroxides in tissues,—radiation injury,[18] parenteral administration of a radiomimetic compound OOA in rats[19] or hypervitaminosis D_2 (Ref. 20), we observed appearance in liver

mitochondria of an ability to deaminate a substrate of DAO cadaverine. These data suggest that the phenomenon of "transformation" (i.e. qualitative alteration in enzymatic properties) of MAO may be realized *in vivo* at least in some pathological conditions.

In this paper we report data dealing mainly with two questions: (1) effect of monoamine oxidase inhibitors (MAOI) on the ability of highly purified MAO from ox or rat liver mitochondria to exhibit qualitative alteration in enzymatic properties under the influence of chemical agents causing oxidation of SH groups in the enzymes, and (2) influence of inhibition of MAO activity *in vivo* on the appearance of an ability in liver mitochondria to deaminate substrates of DAO after parenteral administration of OOA in rats.

MATERIALS AND METHODS

Purification and assays of enzymes

Methods developed in our Institute for solubilization[21] of structure-bound amine oxidases involve treatment of mitochondrial membranes with non-ionic detergents in an alkaline medium created by addition of benzylamine base which, as one of the substrates of MAO, protects these enzymes from inactivation at high pH values. By subsequent fractionation of proteins present in the solubilized material we obtained from liver mitochondria of ox[16] and rat[20] enzyme preparations which, as judged by polyacrylamide gel electrophoresis and values of specific activity, are comparable with the most purified preparations of MAO from the same sources.[10]

In course of purification of MAO we monitored the enzymatic activity by means of a sensitive colorimetric[18,22] method based on formation of a coloured product (absorption maximum at 450 nm) after deamination of *p*-nitrophenylethylamine (HCl). As a unit of activity of MAO we defined the quantity of enzymes catalyzing liberation of 1 nmole of ammonia/min at 37° in 0·2 M potassium phosphate buffer, pH 7·4. Concentration of protein in samples was determined colorimetrically[23] with crystalline beef serum albumin as a standard.

Ox liver MAO. In experiments with highly purified MAO from ox liver samples contained 0·11–0·16 mg of protein of the enzyme preparation purified about 260-fold (specific activity 250–335 units/mg protein) as compared with the homogenate, one of the substrates in saturating concentrations and 0·1 M phosphate buffer, pH 7·8 up to a final volume 2·0 ml. It is essential to determine the values of saturating concentrations of substrates in special experiments; excess of amines causes strong, substrate inhibition of the deamination reactions (cf. ref. 15). Final concentrations of tyramine and histamine in samples were 3·2 and 25 mM, respectively. We incubated the samples in atmosphere of oxygen at 37° during 25–60 min. Within this period of time reactions of deamination of amines follow zero order kinetics. After the incubation period we added trichloroacetic acid (final concentration 5%) into the samples, removed the sediment formed by centrifugation (8000 *g* for 5 min) and in the supernatant fluid determined by means of isothermic diffusion in Conwey units with subsequent nesslerization content of ammonia liberated.

Pretreatment of enzyme preparations with OOA we carried out in presence of saturating concentration of a substrate (3·2 mM tyramine). Samples of OOA containing about $1{\cdot}40 \times 10^{-3}$ mole oxygen/g, as determined by means of iodometric

titration,[6] were prepared by blowing a current of oxygen[6] through chemically pure oleic acid at 60° during 20 hr. After pretreatment of MAO during 120 min at 37° with the OOA we dialyzed samples (48 hr at 4° against four changes of 4 l. portions of 0·004 M phosphate buffer, pH 7·4) up to complete removal[13] of tyramine and OOA.

Preincubation of MAO with Cu^{2+} was carried out during 36 hr at 4° in samples containing in a total volume of 4·0 ml: 3 mM phosphate buffer, pH 7·4, 0·4 mg of protein of the MAO preparation, 0·1 mM of $CuSO_4$, 24 μg of chloroamphenicol. We then added in samples EDTA up to a final concentration 0·1 mM and dialysed the mixture during 12 hr against 300-fold excess of 3 mM phosphate buffer, pH 7·4.

Pretreatment of ox liver MAO with inhibitors (0·1 mM) was carried out for 30 min at room temperature.

Rat liver MAO. Samples contained 0·1 mg of protein of the enzyme preparation purified about 200-fold (specific activity 2000 units/mg protein) as compared with homogenate, one of the substrates in the following saturating concentrations (mM): tyramine—3, 5-HT—5, histamine—10 and 0·1 M potassium phosphate buffer, pH 7·4 up to a total volume of 1·8 ml. Conditions of incubation (50 min), fixation of samples and estimation of activity were as described above.

Dialysis after addition of EDTA in samples containing enzyme pretreated with Cu^{2+} has been carried out with constant stirring during 3 hr. Preincubation with MAOI (0·01 mM)—15 min at room temperature.

Experiments in vivo

We have used white male rats (120–150 g) which fasted within 16–18 hr before the experiment. OOA[$1{\cdot}40 \times 10^{-3}$ mole oxygen/g] was injected intraperitoneally into the rats in gradually increasing amounts (0·08, 0·1, 0·12 and 0·15 ml) during 4 days. Iproniazid was dissolved in 0·9% NaCl, neutralized and injected intraperitoneally in a dose 150 mg/g 16 hr before the first injection of OOA. Second injection of iproniazid was made 72 hr after the first injection of the inhibitor; 0·9% NaCl was injected in control rats. All the animals were sacrificed by decapitation on the 6th day after the first injection of iproniazid. Liver was quickly removed and mitochondria isolated as described previously.[12] Samples, containing in a total fluid volume of 1·8 ml: 0·1 M phosphate buffer, pH 7·4, 4 mg of protein of rat liver mitochondria and one of the substrates in following saturating concentrations (mM): serotonin—5, histamine—10, putrescine—10, L-lysine—10, were incubated during 45 min.

Special chemicals

As substrates we have used tyramine. HCl (T. Schuchardt, GFR), serotonin creatinine sulfate and L-lysine. HCl (Reanal, Hungary), *p*-nitrophenylethylamine. HCl (commercially available Soviet reagent), histamine. 2HCl (Calbiochem, U.S.A.). As MAOI—1-isonicotynyl-2-isopropylhydrazine base (iproniazid, iprazid) synthesised in the All-Union Research Institute of Pharmaceutical Chemistry (Moscow), *N*-methyl-*N*-benzylpropynylamine.HCl (pargyline) and *trans*-2-phenylcyclopropylamine. 1/2 H_2SO_4 (tranylcypromine) both synthesised in the Institute of Organic Synthesis, Academy of Sciences of Latvian SSR (Riga).

RESULTS

Highly purified ox liver mitochondrial MAO readily deaminates tyramine but does not catalyse deamination of histamine (Table 1). Preincubation of the enzyme with low concentrations (0·1 mM) of specific MAOI pargyline, iproniazid, tranylcypromine, which all belong to various classes of chemical compounds but possess one common property—an ability to inhibit activity of MAO, causes complete inhibition of the deamination of tyramine. At the same conditions *p*-chloromercuribenzoate (pCMB) and *N*-ethylmaleimide (NEM) cause partial inhibition of deamination of tyramine by ox liver MAO (Table 1). Pretreatment of the enzyme with OOA in

TABLE 1. EFFECT OF INHIBITORS ON QUALITATIVE ALTERATION IN ENZYMATIC PROPERTIES OF HIGHLY PURIFIED MITOCHONDRIAL MONOAMINE OXIDASE FROM OX LIVER

Treatment of enzyme	Rate of deamination* (nmoles NH_3/mg protein/min)	
	Tyramine	Histamine
Untreated	1250 ± 12	0
Pargyline	0	—
Iproniazid	0	—
Tranylcypromine	0	—
pCMB	418 ± 9	—
NEM	510 ± 11	—
OOA	140 ± 3	59 ± 2
$CuSO_4$	104 ± 7	41 ± 4
OOA + pargyline	0	47 ± 3
OOA + tranylcypromine	0	50 ± 4
OOA + pCMB	132 ± 7	30 ± 6
OOA + NEM	129 ± 5	42 ± 5
Pargyline + OOA	0	0
Iproniazid + OOA	0	0
Tranylcypromine + OOA	0	0
pCMB + OOA	93 ± 7	0
NEM + OOA	112 ± 7	0

* MAO activity is expressed in V_{max} values (nmoles of ammonia liberated per mg protein per min). Results are mean values ± S.E. Means for four to five experiments. For experimental conditions see Materials and Methods.

conditions described in Materials and Methods not only decreases the rate of deamination of tyramine but also induces appearance of a qualitatively new reaction—an ability to deaminate histamine. Treatment with copper ions of the enzyme in aerobic conditions causes similar effect on its enzymatic activity (Table 1). These data are in complete agreement with our previous findings.[13–15] Neither MAOI nor the reagents interacting with SH-groups cause any *qualitative* alteration in the MAO activity.[15] Deamination of histamine by MAO, pretreated with OOA, is resistant towards the effect of MAOI; pCMB and NEM cause partial inhibition of this reaction (Table 1). Preincubation of ox liver MAO with one of MAOI completely prevents appearance of histamine deaminating activity when these enzyme preparations are treated with OOA. This effect, however, is not specific for the MAOI. Even partial inhibition of

MAO activity with pCMB or NEM prevents appearance of histamine deaminating activity after treatment of the enzyme with OOA (Table 1).

Highly purified MAO from rat liver readily deaminates tyramine or 5-HT but does not deaminate histamine (Table 2). Very low concentrations (0·01 mM) of MAOI pargyline and tranylcypromine partially inhibit deamination of the monoamines. Pretreatment of the enzyme with copper ions in aerobic conditions decreases the MAO activity and, at the same time, induces appearance of a qualitatively new reaction—an ability to deaminate histamine. This phenomenon is completely prevented by the MAOI (Table 2).

TABLE 2. EFFECT OF MONOAMINE OXIDASE INHIBITORS ON QUALITATIVE ALTERATION IN ENZYMATIC PROPERTIES OF HIGHLY PURIFIED MITOCHONDRIAL MONOAMINE OXIDASE FROM RAT LIVER

Treatment of enzyme	Rate of deamination* (nmoles NH_3/mg protein/min)		
	Tyramine	5-HT	Histamine
Untreated	743	312	0
Pargyline	35	65	—
Tranylcypromine	124	55	—
$CuSO_4$	148	—	40
Pargyline + $CuSO_4$	—	—	0
Tranylcypromine + $CuSO_4$	—	—	0

* Means for two experiments (with four parallel determinations in each).

After repeated parenteral administration of OOA into rats we observed a decrease in deamination of 5-HT accompanied by appearance of an ability to deaminate histamine, putrescine and L-lysine in liver mitochondria (Table 3). Pretreatment of rats with iproniazid completely prevents these phenomena (Table 3).

TABLE 3. EFFECT OF PRETREATMENT OF RATS WITH IPRONIAZID ON APPEARANCE OF HISTAMINE-, PUTRESCINE- AND L-LYSINE-DEAMINATING ACTIVITIES IN RAT LIVER MITOCHONDRIA AFTER ADMINISTRATION INTO THE ANIMALS OF OXIDIZED OLEIC ACID (OOA)

Treatment of rats	Rate of deamination* (nmoles NH_3/mg protein/min)			
	Serotonin	Histamine	Putrescine	L-lysine
Untreated	11 ± 0·3	0	0	0
Iproniazid	0·8, 0·8 (2)	—	—	—
OOA	4 ± 0·1	1·2 ± 0·1	2·1 ± 0·3	0·5 ± 0·07
Iproniazid + +OOA	0·2 ± 0·05	0	0	0

* Means for three to five experiments.

DISCUSSION

It is well known that MAO deaminates various toxic biogenic amines and that MAOI block these reactions. There are, however, data which are not in complete agreement with these simple concepts. Indirect evidence[24,25] for "multiplicity" of MAO was confirmed by physical separation of MAO deaminating various monoamines.[26–30] MAO is, thus, not a single enzyme (EC 1.4.3.4) deaminating all the biogenic amines.[3] Some MAOI selectively inhibit[31,32] deamination of various monoamines. The "detoxication" function[33] is not a single biological role of MAO. It is obvious that MAO may catalyse formation in course of deamination of biogenic amines of new biologically active substances[3] influencing, for example, the mitochondrial succinate dehydrogenase activity;[34,35] MAOI prevent these effects. Inhibitory effects of monoamines on γ-amylase (α-glucosidase) in rat liver and heart muscle[26] and on cell division in sea urchin eggs[37] are prevented by MAOI. All these data suggest that MAOI *in vivo* may, besides increasing tissue levels of biogenic amines, prevent formation of other biologically active substances (e.g. aldehydes) in course of oxidative deamination of the amines.

Results of the present work show that MAOI prevent both *in vitro* and *in vivo* the process[13–15] of qualitative alteration ("transformation") of enzymatic properties of MAO. Ability of highly purified preparations of soluble bacterial MAO (tyramine oxidase) to exhibit after treatment with Cu^{2+}, *o*-iodosobenzoate or other oxidizing agents qualitatively new ability to catalyze oxidative deamination of L-lysine, putrescine, spermine[17] is prevented by pretreatment of the enzyme with iproniazid.[17]

These data and the results of the present work suggest that the phenomenon of "transformation" (qualitative alteration in enzymatic properties) of MAO does not take place when the active sites of these enzymes are blocked with specific irreversible inhibitors.

It is possible that in the future MAOI may find some new fields of application based on their ability to prevent qualitative alterations in enzymatic properties of MAO *in vivo* in pathological states (for example, in those which like radiation injury or hypervitaminosis D_2 are accompanied by accumulation of lipid peroxides in tissues).

REFERENCES

1. V. Z. GORKIN and S. YA. DAVYDOVA, *Vopr. med Khim.* **16,** 591 (1970).
2. V. Z. GORKIN, *Experientia* **26,** 1234 (1970).
3. V. Z. GORKIN, *Pharmac. Rev.* **18,** 115 (1966).
4. A. L. LEHNINGER, *Physiol. Rev.* **42,** 467 (1962).
5. I. S. SEVERINA and V. Z. GORKIN, *Biokhimiya* **28,** 896 (1963).
6. E. A. RAPAVA, L. B. KLYASHTORIN and V. Z. GORKIN, *Biokhimiya* **31,** 1216 (1966).
7. L. B. KLYASHTORIN, L. I. GRIDNEVA and V. Z. GORKIN, *Biokhimiya* **31,** 167 (1966).
8. L. B. KLYASHTORIN and L. I. GRIDNEVA, *Biokhimiya* **31,** 831 (1966).
9. L. HELLERMAN and V. G. ERWIN, *J. biol. Chem.* **243,** 5234 (1968).
10. B. GOMES, G. NAGUWA, H. G. KLOEPFER and K. T. YASUNOBU, *Archs Biochem. Biophys.* **132,** 28 (1969).
11. V. Z. GORKIN, L. V. TATYANENKO, N. N. SUVOROV and A. D. NEKLYUDOV, *Biokhimiya* **32,** 1036 (1967).
12. V. Z. GORKIN, L. V. TATYANENKO, D. M. KRASNOKUTSKAYA, E. V. PRONINA and L. N. YAKHONTOV, *Biokhimiya* **32,** 510 (1967).
13. V. Z. GORKIN and L. V. TATYANENKO, *Biochem. biophys. Res. Commun.* **27,** 613 (1967).
14. V. Z. GORKIN, L. V. TATYANENKO and T. A. MOSKVITINA, *Biokhimiya* **33,** 393 (1968).
15. V. Z. GORKIN, ZH. I. AKOPYAN, I. V. VERYOVKINA, L. I. GRIDNEVA and L. N. STESINA, *Biokhimiya* **35,** 141 (1970).

16. V. Z. Gorkin, Zh. I. Akopyan and L. N. Stesina, *Vopr. med. Khim.* **16,** 678 (1970).
17. V. A. Yakovlev, V. Z. Gorkin, L. V. Tatyanenko, R. I. Gvozdev, O. I. Lebedeva and L. V. Vorobyov, *Dokl. Akad. SSSR* **197,** 470 (1970).
18. V. Z. Gorkin, Zh. I. Akopyan, E. N. Goncharenko and Yu. B. Kudryashov, *Vopr. med. Khim.* **14,** 538 (1968).
19. Zh. I. Akopyan, V. Z. Gorkin, Yu. B. Kudryashov and O. V. Syomina, *Radiobiologiya* **10,** 826 (1970).
20. Zh. I. Akopyan, N. V. Blazheievich, I. V. Veryovkina, V. Z. Gorkin, O. V. Syomina and V. B. Spirichev, *Int. J. Vitam. Res.* **40,** 497 (1970).
21. Zh. I. Akopyan, I. V. Veryovkina, M. I. Levyant, T. A. Moskvitina, V. Z. Gorkin and V. N. Orekhovich, *Int. J. Protein Res.* **3,** 678 (1971).
22. E. A. Zeller, H. R. Buerki and T. Ishimaru, *Fedn Proc.* **21,** 271 (1962).
23. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
24. G. A. Alles and E. V. Heegaard, *J. biol. Chem.* **147,** 487 (1943).
25. E. Werle and F. Roewer, *Biochem. Z.* **322,** 320 (1952).
26. V. Z. Gorkin, *Nature, Lond.* **200,** 77 (1963).
27. G. G. S. Collins, M. B. H. Youdim and M. Sandler, *FEBS Letters* **1,** 215 (1968).
28. H. C. Kim and A. D'Iorio, *Can. J. Biochem.* **46,** 295 (1968).
29. J. B. Ragland, *Biochem. biophys. Res. Commun.* **31,** 203 (1968).
30. V. Z. Gorkin, *Experientia* **25,** 1142 (1969).
31. V. Z. Gorkin and L. A. Romanova, *Biochem. Pharmac.* **17,** 855 (1968).
32. V. Z. Gorkin and W. N. Orekhovich, *Biochimica Applicata (Parma)* **14,** 343 (1967).
33. B. Blackwell and E. Marley, *Br. J. Pharmac.* **26,** 120, 142 (1966).
34. V. Z. Gorkin and R. S. Krivchenkova, *Vopr. med. Khim.* **13,** 428 (1967).
35. V. Z. Gorkin and R. S. Krivchenkova, *Vopr. med. Khim.* **16,** 678 (1970).
36. V. S. Orlova, V. N. Sinyukhin, I. A. Popova, V. Z. Gorkin and E. L. Rosenfeld, *Biokhimiya* **35,** 1234 (1971).
37. L. N. Markova and G. A. Buznikov, *Dokl. Acad. Nauk SSSR* **192,** 1184 (1970).