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MITOCHONDRIAL MONOAMINE OXIDASE OF RAT LIVER: REVERSIBLE QUALITATIVE ALTERATIONS IN CATALYTIC PROPERTIES

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SUMMARY

1. Preincubation of rat liver mitochondrial membranes under aerobic conditions in the presence of Cu^{2+} (but not of other metal ions) caused a significant decrease in monoamine oxidase (monoamine: oxygen oxidoreductase, EC 1.4.3.4) activity and induced the ability to deaminate substrates of diamine oxidase (diamine: oxygen oxidoreductase (deaminating), EC 1.4.3.6) (histamine and some diamines) and AMP. At the same time, the content of SH groups in the mitochondrial membranes decreased.

2. Preincubation of highly purified rat liver mitochondrial monoamine oxidase in the presence of Cu^{2+} under aerobic conditions decreased the rate of deamination of monoamines and induced the ability to deaminate histamine, some diamines and AMP. These alterations (transformation) in enzymatic properties of monoamine oxidase were accompanied by a decrease in content of SH groups (from 8 to 1 per 10^5 g of protein) in the enzyme. The decrease in content of SH groups was probably due to their partial oxidation. The modified enzyme was insensitive to specific monoamine oxidase inhibitors (pargyline, tranylcypromine), but it was inhibited by some carbonyl reagents (hydroxylamine, isoniazid).

3. Qualitative alterations in catalytic properties of monoamine oxidase and the accompanying decrease in content of SH groups were observed after treatment of the enzyme with oxidized oleic acid.

4. Pretreatment of highly purified rat liver monoamine oxidase with specific irreversible monoamine oxidase inhibitors prevented appearance of the histamine deaminating activity in the course of subsequent incubation in the presence of Cu^{2+}.

5. Pretreatment of highly purified monoamine oxidase with alkylating agents (*N*-ethylmaleimide, iodoacetamide) did not alter the catalytic properties of monoamine oxidase qualitatively, but prevented the development of these alterations in the course of subsequent incubation of the enzyme in the presence of Cu^{2+}.

6. Qualitative alteration in catalytic properties of monoamine oxidase (preincubated in presence of Cu^{2+} or treated with oxidized oleic acid) was partially re-

versed by treatment of the enzyme with reducing agents ($NaBH_4$, reduced glutathione, Na_3AsO_3).

INTRODUCTION

It was established previously[1–3] that treatment of rat liver mitochondria with oxidized oleic acid or preincubation of the mitochondria in presence of Cu^{2+} caused not only a decrease in monoamine oxidase activity but also lead to the appearance of a new ability (never observed in the untreated mitochondrial membranes) to deaminate diamines, histamine and other nitrogenous compounds. Treatment of mitochondrial membranes with oxidizing agents did not induce any diamine deaminating activity if the activity of mitochondrial monoamine oxidase was previously blocked[1,2] by one of the irreversibly acting specific monoamine oxidase inhibitors.

These observations suggested the possibility of an effect of oxidizing agents on mitochondrial monoamine oxidase (monoamine: oxygen oxidoreductase, EC 1.4.3.4) with alteration (transformation) in the substrate and inhibitor specificity of the enzyme[2,3]. It was also suggested[3] that the phenomenon of the transformation of monoamine oxidase may be accompanied by reversible oxidation of SH groups of the enzyme.

In order to investigate these possibilities further, we studied the effects of some oxidizing agents on the catalytic properties and content of SH groups in highly purified preparations of rat liver monoamine oxidase.

MATERIALS AND METHODS

Animals

White male rats (180–200 g), which were fasted for 18 h before the experiments, were used.

Enzyme preparations

Methods for isolation of rat liver mitochondria[4] and mitochondrial membranes[5] as well as a procedure for solubilization and purification[6] of monoamine oxidase from rat liver mitochondrial membranes were described previously. The enzyme was prepared in a soluble form, without significant inactivation, by treatment of mitochondrial membranes with a non-ionic detergent in a strongly alkaline medium containing benzylamine (a substrate of monoamine oxidase), which protected the enzyme from inactivation by alkali[6]. By means of subsequent $(NH_4)_2SO_4$ fractionation and treatment with Al_2O_3, enzyme preparations were obtained which were highly purified (cf. ref. 7), as evidenced by electrophoresis in polyacrylamide gel and an increase in specific activity (about 45-fold as compared with mitochondrial suspension).

Reagents

The following were used without further purification: Na_3AsO_3 (Chemapol, Czechoslovakia), $CaCl_2$, $CoCl_2$, $CuCl_2$, $ZnSO_4$, $FeSO_4$, $Fe_2(SO_4)_3$, $MnSO_4$, $CdSO_4$ (all of special purity for spectral analysis); monoamine oxidase inhibitors: pargyline (*N*-methyl-*N*-benzylpropynylamine·HCl; presented by Dr. K. F. Gey, F. Hoffmann-

La Roche and Co, Basle, Switzerland), SU-11739 (*N*-methyl-*N*-2-propynyl-1-indanamine[8,9]; presented by Dr. A. J. Plummer, CIBA Pharm. Co., Summit, N.J., U.S.A.), E-250 (DL-phenylisopropylmethylpropynylamine·HCl[10,11] presented by Dr. K. Magyar, Institute of Pharmacology, University Medical School, Budapest, Hungary), chlorgyline, M and B 9302 (*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)propynylamine·HCl[12,13]; presented by Dr. H. J. Barber, May and Baker, Ltd, Dagenham, Essex, England), tranylcypromine (*trans*-2-phenylcyclopropylamine·0.5 H_2SO_4; synthesized in the Institute of Organic Synthesis, Academy of Sciences of Latvian SSR, Riga), 51641 (*N*-2-(*o*-chlorophenoxy)ethylcyclopropylamine[14]; presented by Dr. R. W. Fuller, The Lilly Research Laboratories, Indianapolis, Ind., U.S.A.). Sources and degrees of purification of other reagents used were stated previously[3].

Oleic acid was oxidized at 60° for 48 h in a constant current of oxygen. The content of oxidation products was estimated polarographically and iodometrically. Samples of the oxidized oleic acid, characterized in this way, were kindly donated by Professor Yu. B. Kudryashov (Moscow University). Samples of oxidized oleic acid used in our experiments contained about 1 nmole of O_2 per g of oleic acid. Stable 4% emulsions of the oxidized oleic acid were prepared by rapid mixing (5000 rev./min, 15 min) of samples of the acid with 0.9% NaCl, which contained 8% ethanol and 1.4% of a non-ionic detergent OP-10 (a Soviet non-ionic detergent which could be replaced by Triton X-100).

Liberation of ammonia

Rates of enzymatic reactions were usually evaluated by measuring liberation of ammonia during the incubation of enzyme preparations with amines. Samples contained either 1.5 mg of protein of mitochondrial membranes or 0.06–0.1 mg of protein of highly purified enzyme preparation, one of the substrates in optimal concentration (experimentally determined) and 0.1 M potassium phosphate buffer (pH 7.4) in a total volume of 1.8 ml. Samples were incubated at 37° in oxygen for 45 min. During this period there was a linear increase in the quantity of ammonia liberated. After addition of trichloroacetic acid (final concentration 5%), the content of ammonia in protein-free supernatants was determined by means of isothermic diffusion and subsequent Nesslerization[1–3] or treatment with dichlorocyanurate reagent[15]. The latter reaction, which exceeds in sensitivity the convential Nessler reaction about 20-fold, was used only when very small amounts of ammonia had to be measured.

Consumption of oxygen

Measurements were carried out in the Institute of Chemical Physics, Academy of Sciences of the U.S.S.R. by Dr. R. I. Gvozdev. Use was made of a highly sensitive glass manometric vessel[16] with a thin sickle-shaped membrane, displacement of which was recorded by means of an inductive sensor connected with a two channel pressure indicator and a recorder. The assembly allowed accurate measurement and continuous recording of comparatively small changes in pressure of the gaseous phase in the presence of liquid phase.

Content of H_2O_2

Quantitative determinations of content of hydrogen peroxide in samples was carried out by means of a highly sensitive colorimetric method based on the oxidation of phenolphthalin into phenolphthalein in alkaline medium[17].

Content of protein

Content of protein was measured as described by LOWRY *et al.*[18] using crystalline beef serum albumin (Koch-Light) as a standard.

Content of SH groups

Content of SH groups was measured colorimetrically with 5,5'-dithiobis-(2-nitrobenzoic acid) as described by ELLMAN[19]. The spectrophotometric procedure of BOYER[20] with *p*-chloromercuribenzoate was employed in our previous work[21] as an independent technique for the estimation of sulfhydryl groups. Results obtained by means of both methods were identical in all the experiments.

Detergent concentration

To measure the content of a non-ionic detergent in samples, use was made[5] of a known property of these detergents to alter the colour of aqueous solutions of indicators. At pH >7.5 the absorption maximum of bromthymol blue solution in the presence of the detergent changes from 623 to 425 nm and the concentration of detergent is proportional in a certain range to the decrease in absorbance at 623 nm.

Content of histamine

Content of histamine in samples was measured by Dr. I. L. Vaisfeld (Academy of Scienes of the U.S.S.R.) who used a fluorimetric technique with *o*-phthalic aldehyde[22].

RESULTS

Effect of Cu^{2+}

Preincubation of mitochondrial membranes or highly purified monoamine oxidase from rat liver in presence of $CuSO_4$ decreased the monoamine oxidase activity and, at the same time, induced a qualitatively new ability to deaminate some nitrogenous compounds (histamine, putrescine, cadaverine, lysine, AMP) which are not the substrates of monoamine oxidase (Table I). The use of $CuCl_2$, in place of $CuSO_4$, gave similar results.

In experiments with the mitochondrial membranes (Table I) samples contained 2.4 ml (36 mg of protein) of suspension of mitochondrial membranes, $CuSO_4$ (final concentration 1 mM), chloroamphenicol (5 μg/ml; added to prevent growth of microorganisms) and 0.05 M potassium phosphate buffer (pH 7.4) to a total volume of 42 ml. Samples were incubated at 4° for 4 days. EDTA (final concentration 1 mM) was then added and the samples were centrifuged (25 000 × *g*, 15 min). The precipitate obtained was suspended in 20 ml of 10 mM potassium phosphate buffer (pH 7.4). Samples for assay of enzymatic activity contained 1.6 mg of protein, one of the substrates and 0.1 M potassium phosphate buffer (pH 7.4) in a total volume of 1.8 ml.

TABLE I

EFFECT OF PREINCUBATION WITH $CuSO_4$ OF RAT LIVER MITOCHONDRIAL MEMBRANES AND HIGHLY PURIFIED MONOAMINE OXIDASE ON DEAMINATION OF SOME NITROGENOUS COMPOUNDS

Conditions of preincubation and assay of activity are described in the text. Average values (from the results of 4–8 assays) show the rate of deamination in nmoles of ammonia liberated per mg of protein per min.

Substrate	*Concn. (mM)*	*Mitochondrial membranes*		*Purified enzyme*	
		Control	Cu^{2+}	*Control*	Cu^{2+}
Tyramine	3	22	4	282	71
Serotonin	5	10	4	–	–
Histamine	10	0	4	0	31
Putrescine	10	0	2	0	19
Cadaverine	10	0	2	0	22
L-Lysine	15	0	2	0	25
AMP	10	0	2	0	22

In experiments with purified monoamine oxidase (Table I) samples contained 0.35 mg of protein of highly purified rat liver mitochondrial monoamine oxidase, $CuSO_4$ (final concentration 0.1 mM), chloroamphenicol (5 μg/ml) and 0.05 M potassium phosphate buffer (pH 7.4) to a total volume 14 ml. Samples were incubated at 4° for 36 h. EDTA (final concentration 0.1 mM) was then added and samples were dialyzed against a 400-fold excess of 3 mM potassium phosphate buffer (pH 7.4) at 4° for 20 h. Samples for assay of enzymatic activity contained 0.08 mg of protein, one of the substrates and 0.1 M potassium phosphate buffer (pH 7.4) in a total volume of 1.8 ml.

Incubation of enzyme preparations in the presence of Cu^{2+} was accompanied by a decrease in content of SH groups from 8 to 1 per 10^5 g of protein. Incubation of the enzyme preparations under anaerobic conditions (in atmosphere of nitrogen) in the presence of Cu^{2+} decreased the content of SH groups only to 4 per 10^5 g of protein and did not induce the histamine deaminating activity.

Incubation of mitochondrial membranes in the presence of $CoCl_2$, $ZnSO_4$, $MnSO_4$, $CaCl_2$, $FeSO_4$, $Fe_2(SO_4)_3$ or $CdSO_4$ (in concentrations equimolar to those of $CuSO_4$ or $CuCl_2$) did not induce histamine deaminating activity; the content of SH groups in these experiments decreased to about 4 per 10^5 g of protein. Incubation of the enzyme preparations in presence of Cd^{2+} (but not of other metal ions) caused a decrease in deamination of serotonin by 40% but did not alter the rate of tyramine deamination. These observations are in agreement with our previous data[23] which showed that deamination of tyramine was completely inhibited when mercaptide-forming reagents blocked five SH groups per 10^5 g of protein in partially purified[24] rat liver monoamine oxidase, but complete inhibition of serotonin deamination was achieved when only three SH groups per 10^5 g of protein were blocked. It is known that Cd^{2+} reacts with vicinal SH groups in proteins[25]. This suggests that SH groups of this kind participate in the enzymatic deamination of serotonin.

Properties of histamine deaminating activity

Thermolability. Decrease in histamine deaminating activity by 45% was

observed after suspensions of mitochondrial membranes, preincubated with Cu^{2+}, were kept at 70° for 15 min. Heating of the suspensions at 100° for 5 min completely destroyed the histamine deaminating activity.

Substrate and enzyme concentration. In samples, which contained enzyme preparations preincubated with Cu^{2+}, the rate of histamine deamination gradually increased with an increase in the substrate concentration (from 5 to 18 mM). Further increase in concentration of histamine (to 25 mM and higher) inhibited the liberation of ammonia. At a constant substrate concentration (18 mM) increase in content of enzyme preparations in samples was accompanied by linear increase in quantities of ammonia liberated.

pH optimum. At pH 7.4 there was a distinct pH optimum for deamination of histamine by mitochondrial membranes, preincubated with Cu^{2+} (Fig. 1).

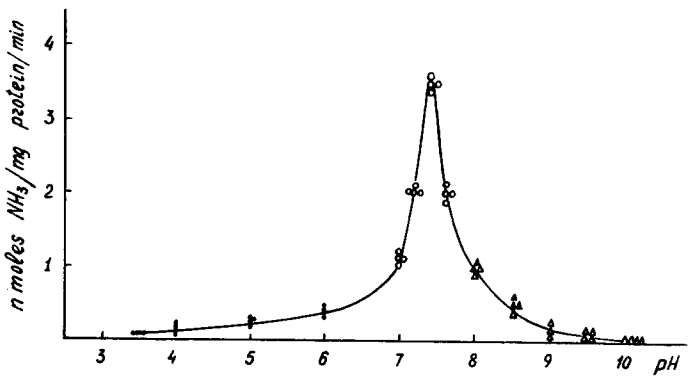

Fig. 1. Effect of pH on the rate of deamination of histamine by rat liver mitochondrial membranes, preincubated with $CuSO_4$. Rate of the reaction at pH 3.5–6.5 was measured in 0.2 M acetate buffer (●), at pH 7.0–7.8 in 0.2 M phosphate buffer (○), at pH 8.0–10.0 in 0.2 M borate buffer (△). Samples contained 1.7 mg of protein of mitochondrial membranes preincubated with $CuSO_4$ as described in the text, histamine (18 mM) and one of the buffer solutions in a total volume of 1.8 ml. Each point shows the result of a single assay.

Kinetics and stoichiometry. A linear increase in the quantity of liberated ammonia with an increase in duration of incubation of enzyme preparations (which were preincubated in the presence of Cu^{2+}) with histamine was observed during the first 30 min of incubation. An observed decrease in the rate of this reaction with longer incubation was probably caused by the effect of H_2O_2 accumulating in samples, which did not contain added catalase. During a standard incubation period the decrease in content of histamine in the samples was about 40% of the amount added. Consumption of 1 mole of oxygen and formation of 1 mole of H_2O_2 per mole of ammonia liberated was observed in the course of the histamine deaminating reaction, which is in agreement with an equation of oxidative deamination.

Effect of inhibitors. The histamine deaminating activity of rat liver mitochondrial membranes or highly purified monoamine oxidase, preincubated in presence of Cu^{2+}, was characterized by insensitivity to specific monoamine oxidase inhibitors (pargyline, tranylcypromine); significant inhibition of this reaction was caused by some diamine oxidase inhibitors (aminoguanidine, isoniazid, *etc.*) (Table II).

In the experiments, presented in Table II, preincubation of enzyme prepara-

TABLE II

EFFECT OF SOME INHIBITORS ON DEAMINATION OF HISTAMINE BY RAT LIVER MITOCHONDRIAL MEMBRANES AND PURIFIED MONOAMINE OXIDASE, PREINCUBATED IN PRESENCE OF Cu^{2+}

Experimental conditions are described in the text. Average values (from the results of 4–8 assays) show the inhibition of histamine deamination (% of the rate of this reaction in control samples, which did not contain the inhibitors).

Inhibitor	*Mitochondrial membranes*	*Purified enzyme*
Aminoguanidine	22	23
Hydroxylamine	63	59
Isoniazid	50	51
Pargyline	2	5
Tranylcypromine	2	4

tions in presence of $CuSO_4$ was carried out under conditions described above (Table I and the corresponding text). Final concentrations of the monoamine oxidase inhibitors in the samples were 0.1 mM. Preincubation of the inhibitors with the enzyme preparations before addition of histamine (18 mM) was carried out at room temperature for 15–20 min (cf. ref. 26). Other inhibitors (Table II) were used in final concentrations of 1 mM. In control samples, which did not contain inhibitors average liberation of ammonia (due to deamination of histamine under standard conditions of incubation) in nmoles/mg protein per min was 53 with purified monoamine oxidase and 5 with mitochondrial membranes.

Preincubation of highly purified monoamine oxidase with pargyline or tranylcypromine (1 mM) at room temperature for 15 min ensured complete inhibition of monoamino oxidase activity (substrates tyramine or serotonin) (Table III).

TABLE III

EFFECT OF SPECIFIC INHIBITORS ON THE ACTIVITY OF HIGHLY PURIFIED RAT LIVER MITOCHONDRIAL MONOAMINE OXIDASE

Samples contained 0.05 mg of protein of the enzyme preparation, one of the inhibitors (preincubation at room temperature for 15–30 min), tyramine (6 μmoles) or serotonin (10 μmoles) and 0.2 M potassium phosphate buffer (pH 7.4) in a total volume of 1.8 ml. In control samples (without the inhibitors) under standard conditions of incubation liberation of 4.4 or 1.8 μmoles of ammonia (substrates tyramine or serotonin, respectively) was observed.

Inhibitor	*Concn. (mM)*	*Inhibition of deamination (%)*	
		Tyramine	*Serotonin*
Pargyline	0.01	97	80
	0.001	83	62
SU-11739	0.001	90	71
	0.0001	80	62
E-250	0.01	94	78
	0.001	90	64
Chlorgyline	0.01	73	63
Tranylcypromine	0.01	84	84
	0.001	56	57
51641	0.01	86	100
	0.0001	23	63

Experiments with highly purified rat liver monoamine oxidase (Table III) confirmed the ability of pargyline to inhibit deamination of tyramine at lower concentrations, than those required for inhibition of deamination of serotonin[27]. Similar effects were observed with some other derivatives of propynylamine SU 11739 (refs. 8, 9), E-250 (refs. 10, 11) and, to a lesser degree, with chlorgyline[12,13]. In our experiments with highly purified rat liver mitochondrial monoamine oxidase, the plot of percentage inhibition against concentration of chlorgyline showed a simple sigmoid curve, but did not reveal the characteristic pattern ("a pair of sigmoid curves joined by a horizontal section where the inhibition is invariant"[12]) observed in experiments with rat brain mitochondrial fraction[12]. Tranylcypromine, as might be expected on the basis of previous experiments[28], inhibited the deamination of tyramine and serotonin to the same degree. However, another cyclopropylamine derivative 51641 (ref. 14) selectively inhibited the deamination of serotonin.

The histamine deaminating activity was not induced by incubation with Cu^{2+} of highly purified monoamine oxidase, which had been pretreated with specific monoamino oxidase inhibitors. These observations are in complete agreement with the assumption[1-3] that one of conditions of transformation of monoamine oxidase is the presence of an unblocked catalytic center in the enzyme.

Pretreatment of monoamine oxidase with reagents, which alkylate the SH groups, prevented the ability of the enzyme to show qualitative alterations (transformation) in catalytic properties after preincubation in presence of Cu^{2+} (Table IV).

In the experiments presented in Table IV, samples which contained in a total volume of 10 ml (0.1 M potassium phosphate buffer, pH 7.4) 0.8 mg of protein of highly purified monoamine oxidase and one of the alkylating reagents (5 mM *N*-ethylmaleimide, 1 mM iodoacetamide, 5 mM sodium iodoacetate), were incubated at room temperature for 1 h. Preincubation of aliquots in the presence of $CuSO_4$, assay of enzymatic activity and estimation of SH groups were carried out as described above.

TABLE IV

EFFECT OF ALKYLATING AGENTS ON APPEARANCE OF HISTAMINE DEAMINATING ACTIVITY IN HIGHLY PURIFIED RAT LIVER MITOCHONDRIAL MONOAMINE OXIDASE PREINCUBATED WITH Cu^{2+}

Experimental conditions are described in the text. Average values from the results of 2–4 assays are presented.

Expt. No.	*Treatment of enzyme*	*Deamination (nmoles/mg protein per min)*		*SH groups (M/10⁵ g protein)*
		Tyramine	*Histamine*	
I	—	670	0	8
	$CuSO_4$	130	60	1
II	—	1340	0	8
	N-Ethylmaleimide	250	0	2
	Iodoacetamide	1200	0	3
	Iodoacetate	1335	0	6
III	—	900	0	8
	N-Ethylmaleimide + $CuSO_4$	50	0	1
	Iodoacetamide + $CuSO_4$	83	0	1
	Iodoacetate + $CuSO_4$	91	32	2

As shown in Table IV, transformation of monoamine oxidase by incubation of the enzyme with Cu^{2+} was prevented by pretreatment of the enzyme with *N*-ethylmaleimide, which considerably decreased the content of SH groups in the enzyme preparations and inhibited deamination of tyramine but did not induce the histamine deaminating activity. Similar effects were shown by iodoacetamide. However iodoacetate, which caused only a slight reduction in the content of SH groups in the monoamine oxidase preparations and had practically no effect on the deamination of tyramine, did not prevent the appearance of histamine deaminating activity in monoamine oxidase preparations incubated in presence of Cu^{2+}.

Reversibility. Partial restoration of the content of SH groups and of values of the tyramine deaminating activity with simultaneous inhibition of the induced histamine deaminating activity was caused by treatment with various reducing reagents ($NaBH_4$, Na_3AsO_3, reduced glutathione) of highly purified monoamine oxidase preparations, which had been incubated in the presence of Cu^{2+} (Table V).

TABLE V

EFFECT OF REDUCING AGENTS ON SOME PROPERTIES OF HIGHLY PURIFIED RAT LIVER MONOAMINE OXIDASE PREINCUBATED WITH Cu^{2+}

Average results from 4 assays.

Expt. No.	*Treatment of enzyme*	*Deamination (nmoles/mg protein per min)*		*SH groups ($M/10^5$ g protein)*
		Tyramine	*Histamine*	
I	—	675	0	8
	$CuSO_4$	130	60	1
	$CuSO_4$ + GSH	550	0	6
	$NaBH_4$	465	0	–
	$CuSO_4$ + $NaBH_4$	345	0	6
II	—	1135	0	–
	Na_3AsO_3	1030	0	–
	$CuSO_4$	253	116	–
	$CuSO_4$ + Na_3AsO_3	655	0	5

In the experiments presented in Table V, samples contained 0.05 mg of protein and 0.1 M potassium phosphate buffer (pH 7.4) in a total volume of 1.8 ml. Preincubation with $CuSO_4$ was carried out as described above. Reduced glutathione (GSH) was added in the samples indicated up to 3 mg per mg of protein; the samples were dialyzed before estimation of the SH groups. Control experiments (not presented in Table V) showed that GSH under these experimental conditions did not influence the rate of enzymatic deamination of tyramine. $NaBH_4$ (3.3 mg/mg protein) after incubation at room temperature (pH 7.4) for 10 min did not reduce 5,5'-dithiobis-(2-nitrobenzoic acid) (*cf.* ref. 29). Reduction with Na_3AsO_3 (0.4 mM) was carried out at pH 7.8 in 0.1 M potassium phosphate buffer. Under these conditions Na_3AsO_3 did not reduce 5,5'-dithiobis-(2-nitrobenzoic acid).

Effect of oxidized oleic acid

Treatment of highly purified rat liver monoamine oxidase with oxidized oleic

TABLE VI

EFFECT OF TREATMENT OF HIGHLY PURIFIED RAT LIVER MONOAMINE OXIDASE WITH OXIDIZED OLEIC ACID ON DEAMINATION OF SOME NITROGENOUS COMPOUNDS

Average results of 4–8 assays.

Substrate	*Optimal concn. (mM)*	*Deamination (nmoles/mg protein per min)*	
		Native enzyme	*Treated with oxidized oleic acid*
Tyramine	3	775	373
Serotonin	5	268	107
Tryptamine	2	261	74
Dopamine	3	393	140
Histamine	10	0	80
Putrescine	10	0	49
Cadaverine	10	0	47
L-Lysine	15	0	39
AMP	10	0	113

acid caused a significant decrease in deamination of monoamines (tyramine, serotonin, tryptamine, dopamine) and, at the same time, induced the ability to deaminate some nitrogenous compounds (histamine, putrescine, cadaverine, lysine, AMP), which are not the substrates of monoamine oxidase.

In experiments presented in Table VI, the samples contained in a total volume 12.8 ml (0.2 M potassium phosphate buffer, pH 7.4) 1 mg of protein of the highly purified monoamine oxidase, 6 μmoles of tyramine (added for protection of active sites of monoamine oxidase), oxidized oleic acid (0.95 mmoles of O_2 per g of acid) in a final concentration 10 mM. Samples were incubated for 2 h at 37° in oxygen and then dialyzed against 400-fold excess of 1 mM potassium phosphate buffer (pH 7.4) for 36 h to remove oxidized oleic acid and tyramine. Samples for assay of enzymatic activity contained 0.075 mg of protein, 0.2 ml of 0.2 M potassium phosphate buffer

TABLE VII

EFFECT OF REDUCING AGENTS ON SOME PROPERTIES OF HIGHLY PURIFIED RAT LIVER MONOAMINE OXIDASE PRETREATED WITH OXIDIZED OLEIC ACID

Samples contained 0.07 mg of protein. Experimental conditions are described in the text. A typical experiment is presented.

Treatment of enzyme	*Deamination (nmoles/mg protein per min)*		*SH groups (M/10⁵ g protein)*
	Tyramine	*Histamine*	
—	740	0	8.0
Oxidized oleic acid	361	78	2.3
Oxidized oleic acid + GSH	593	0	6.2
$NaBH_4$	435	–	–
Oxidized oleic acid + $NaBH_4$	316	0	5.8
Na_3AsO_3	725	–	–
Oxidized oleic acid + Na_3AsO_3	522	0	5.5

(pH 7.4), one of the substrates and water in a total volume of 1.8 ml. Conditions of incubation were described in MATERIALS AND METHODS.

In those experiments, in which incubation of monoamine oxidase with oxidized oleic acid failed (for various reasons) to inhibit significantly the deamination of monoamines, no appearance of deamination of diamine oxidase substrates or of AMP was observed.

Treatment of monoamine oxidase with oxidized oleic acid was accompanied by a decrease in content of SH groups in the enzyme preparations; addition of reducing agents partially restored the content of SH groups and (when "mild" reducing reagents were used) the tyramine deaminating activity to the initial level and simultaneously inhibited the induced histamine deaminating activity (Table VII).

Studies of the effects of inhibitors on the histamine deaminating activity of highly purified rat liver monoamine oxidase pretreated with oxidized oleic acid gave results, which were quite similar to those shown in Table II: specific monoamine oxidase inhibitors did not inhibit deamination of histamine, but some inhibitors of diamine oxidase caused inhibition of this reaction.

Tyramine did not cause competitive inhibition of histamine deamination by highly purified monoamine oxidase pretreated with oxidized oleic acid; in the presence of both amines, liberation of ammonia was equal to the sum of the values obtained with each of the amines alone (Table VIII).

TABLE VIII

EFFECT OF TYRAMINE ON DEAMINATION OF HISTAMINE BY HIGHLY PURIFIED RAT LIVER MONOAMINE OXIDASE PRETREATED WITH OXIDIZED OLEIC ACID

Conditions of pretreatment of the enzyme with oxidized oleic acid are described in the text. Samples for assay of enzymatic activity contained 0.1 mg of protein, 0.2 ml of 0.2 M potassium phosphate buffer (pH 7.4), substrates and water in a total volume of 1.8 ml. Mean values ± standard error from 4 assays are presented.

Substrate	*Concn. (mM)*	*Liberation of ammonia (nmoles/mg protein per min)*
Histamine	18	50 ± 2
Tyramine	6	221 ± 4
Histamine + tyramine	18 + 6	274 ± 7

DISCUSSION

The results obtained show that rat liver mitochondrial monoamine oxidase possesses a property which was not recognized before. This property is the ability to undergo, under certain experimental conditions (preincubation with Cu^{2+}, treatment with oxidized oleic acid), reversible qualitative alterations (transformation) in catalytic properties. Under the conditions indicated above, we observed a decrease in the monoamine oxidase activity and the simultaneous appearance of a qualitatively new ability to deaminate some nitrogenous compounds (histamine, putrescine, cadaverine, lysine, AMP), which are not substrates of monoamine oxidase. The deamination of histamine was an oxidative reaction, but AMP underwent hydrolytic deamination. Qualitative alterations (transformation) in catalytic properties of another

oxidative enzyme with an induction of the ability to catalyze hydrolytic reactions were found in studies of glyceraldehyde-3-phosphate dehydrogenase[30]. In a previous publication[3] we have mentioned the transformation of lipoamide dehydrogenase into an NADH–dichlorophenolindophenol reductase[49] by treatment of purified lipoamide dehydrogenase with trace amounts of Cu^{2+}.

Solutions of highly purified rat liver monoamine oxidase used in our experiments contained about 5% of a non-ionic detergent OP-10. Solutions of highly purified beef liver monoamine oxidase[6] contained about 0.75% of Triton X-100. Treatment of this latter enzyme with oxidized oleic acid, H_2O_2 or preincubation with Cu^{2+} also decreased the monoamine oxidase activity and induced an ability to deaminate some nitrogenous compounds (cadaverine, putrescine, spermine, lysine)[31] and AMP[32] which are not the substrates of monoamine oxidase. We have demonstrated previously that there are striking species differences in the sensitivity of beef and rat liver mitochondrial monoamine oxidase to the inhibitory effects of harmine[33] and other β-carboline derivatives[34], structural analogues of tryptamine[35], and some tricyclic dyes[36]. As shown in the present work, these species differences apparently did not influence the ability of mitochondrial monoamine oxidase to undergo reversible qualitative alterations in catalytic properties when these enzymes were treated with some oxidizing agents.

The question arises whether the alteration in catalytic properties of monoamine oxidase could be an artifact due to the presence of detergents in the enzyme preparations. An unequivocal answer to this question was obtained from experiments with soluble bacterial monoamine oxidase which was isolated and purified[37] without the use of detergents. It was shown[21,38] that preincubation of this enzyme in presence of $CuSO_4$, treatment of the enzyme with *o*-iodosobenzoate or with ergosterol peroxide[21] sharply decreased the rate of tyramine deamination and induced, at the same time, an ability to deaminate lysine, putrescine, spermine[38] and AMP[21].

Elucidation of the molecular basis for transformation of monoamine oxidase requires special investigation. We have noted a significant decrease in the content of SH groups in monoamine oxidase preparations incubated in the presence of Cu^{2+}, when qualitative alterations in the enzymatic properties occurred. These data suggested the possibility that there is a correlation between reversible qualitative alterations in catalytic properties of monoamine oxidase and reversible oxidation of SH groups of the enzymes. The following facts favour this hypothesis: (1) Alteration in catalytic properties of monoamine oxidase preincubated with Cu^{2+} took place only when the preincubation was carried out under aerobic conditions. (2) Reagents, which are able to alkylate the SH groups or to form mercaptides, significantly decreased the content of SH groups in monoamine oxidase preparations but did not induce qualitative alterations in their enzymatic properties. (3) Pretreatment of monoamine oxidase preparations with alkylating reagents, which irreversibly blocked some of the SH groups, prevented qualitative alterations in enzymatic properties of monoamine oxidase under conditions of subsequent treatment of monoamine oxidase with oxidizing reagents (*cf.* ref. 31). (4) Qualitative alterations in catalytic properties of monoamine oxidase were induced by treatment of monoamine oxidase with oxidized oleic acid and oxidizing agents, which caused, at the same time, a significant decrease in the content of SH groups in the enzyme preparations. (5) Various reducing agents added to samples of the transformed monoamine oxidase caused more or less complete

restoration of the content of SH groups and of the catalytic properties towards the initial level.

In weakly alkaline medium arsenite possesses the properties of a mild reducing agent, which can reduce only derivatives of sulphenic acid SOH, but not those of sulphonic acid or disulphide bonds[39]. If this is correct, then the partial restoration by arsenite of the content of SH groups oxidized in course of pretreatment of monoamine oxidase with oxidized oleic acid or under conditions of preincubation of monoamine oxidase with Cu^{2+}, might suggest that the products of oxidation of SH groups in monoamine oxidase under our experimental conditions are not only the disulphide bonds, as thought before[2], but probably also stabilized derivatives of sulphenic acid. Similar conclusions were reached in studies of the effects of some oxidizing agents on the SH groups of glyceraldehyde-3-phosphate dehydrogenase[39–42].

At the present time we are interested in exploring experimental approaches to the question whether the qualitative alterations in enzymatic activities, which were observed in experiments with purified monoamine oxidase or mitochondrial membranes, occur in whole organisms in pathological states.

Mitochondria, isolated from the liver of rats with radiation injury[43,44] or hypervitaminosis D (ref. 45), exhibited not only a decrease in monoamine oxidase activity but also acquired a qualitatively new ability to deaminate some nitrogenous compounds (histamine, cadaverine, lysine, AMP), which are not substrates of monoamine oxidase. The content of lipid peroxides, which resemble oxidized oleic acid in their chemical nature and biological effects, increased in tissues of animals under these pathological conditions[43–45]. Parenteral injections into rats of oxidized oleic acid caused a decrease in monoamine oxidase activity of liver mitochondria and induced the qualitatively new ability to deaminate substrates of diamine oxidase and some other nitrogenous compounds[44]. These phenomena were not observed in rats pretreated with monoamine oxidase inhibitors[46].

We could not observe any decrease in the rate of deamination of monoamines or appearance of an ability to deaminate other nitrogenous compounds in the liver of rats which were subjected to repeated parenteral injections of $CuSO_4$ in doses required to increase significantly the concentration of Cu^{2+} in liver[47]. However, we found distinct histamine deaminating activity in biopsy samples obtained by means of liver punctures from two patients with hepatolenticular degeneration. This disease is accompanied by an increase in concentration of copper in liver[48]. No histamine deaminating activity was observed in liver of four men, who did not suffer from hepatolenticular degeneration.

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