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INDUCED APPEARANCE OF ADENYLATE-DEAMINATING ACTIVITY IN HIGHLY PURIFIED BOVINE LIVER MITOCHONDRIAL MONOAMINE OXIDASE

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SUMMARY

1. Treatment of highly purified preparations of bovine liver mitochondrial monoamine oxidase (monoamine:O_2 oxidoreductase (deaminating), EC 1.4.3.4) with chemical reagents (*e.g.* oxidizing agents), which caused reversible qualitative alterations in catalytic properties of monoamine oxidases, induced appearance in the enzyme preparations of an ability to catalyze hydrolytic deamination of AMP (ADP and ATP, contrary to other nucleotides or nucleosides studied, were also deaminated).

2. Enzymatic deamination of AMP by highly purified bovine liver mitochondrial monoamine oxidase, pretreated with oxidized oleic acid, was inhibited by carbonyl reagents (isoniazid, hydroxylamine). These reagents did not inhibit deamination of AMP by purified AMP deaminase from rabbit skeletal muscles.

3. Qualitative alteration in catalytic properties of monoamine oxidase (accompanied by the appearance of AMP-deaminating activity in highly purified monoamine oxidases) probably took place also *in vivo* in experimental pathological states characterized by accumulation of lipid peroxides in tissues.

INTRODUCTION

It has been shown previously that catalytic properties of amine oxidases may undergo reversible qualitative alterations[1–7]. The appearance in purified monoamine oxidases (monoamine:O_2 oxidoreductase (deaminating), EC 1.4.3.4) of an AMP-deaminating activity has been observed[2,5,7]. These data suggest that an interrelationship exists, previously unrecognized, between the amine oxidizing and AMP-deaminating activities.

Data on the nature and properties of the AMP-deaminating activity, which appeared in highly purified bovine liver mitochondrial monoamine oxidase after treatment of the enzyme with some oxidizing reagents, are presented in this paper. We report also some data, which suggest that the reversible modification of the

properties of monoamine oxidases accompanied by the induced appearance of AMP-deaminating activity, may take place *in vivo* under certain pathological conditions.

MATERIALS AND METHODS

Animals

White male rats (180–200 g) were used. Animals with hypervitaminosis D_2 (ref. 8) were provided by Drs V. B. Spirichev and N. V. Blazhevevich. Methods of X-ray irradiation and administration of oxidized oleic acid to rats have been described previously[9,10].

Enzyme preparations

Methods of isolation and purification to electrophoretic homogeneity of monoamine oxidase from bovine liver mitochondria have been described previously[3]. AMP deaminase from rabbit skeletal muscles (Schmidt AMP deaminase, EC 3.5.4.6) was kindly presented by Sigma (U.S.A.). The enzyme preparation contained about 2.2 μmole units of the enzymatic activity in 1 ml of suspension (in 1 M KCl).

Reagents

8-Bromoadenylic acid was synthesized in the Department of Organic Chemistry of our Institute by Dr A. I. Tochilkin. Sources of other reagents were indicated in previous publications[3,7].

Liberation of ammonia

When the rates of enzymatic reactions were estimated by measurements of ammonia liberated in the course of incubation of the purified enzyme preparations with one of the nitrogenous components, the samples (final volume 1.8 ml) usually contained 0.05 mg of protein, one of the substrates at the optimal concentration (which was found experimentally) and a suitable buffer solution. The conditions of incubation, fixation of samples and estimation of ammonia have been described previously[7].

Decrease in content of AMP

The initial rate of the AMP deaminase reaction was estimated by measuring the decrease in content of AMP in thermostated cells of an SF-4a spectrophotometer by following the decrease in absorbance at 257 nm.

Formation of inosinic acid

The separation of nucleotides in incubated samples was carried out by Drs L. B. Rebrov and V. L. Kozeltzev who used the method of thin-layer chromatography in DEAE-cellulose. A suspension of 0.95 g DEAE-cellulose and 0.24 g cellulose powder in 12 ml of water was used as the immobile phase; 0.02 M HCl as the solvent. The chromatographic separation was carried out for 35 min. The nucleotide-containing parts of the chromatograms (dark spots in ultraviolet light) were scratched out and extracted with 0.5 M NaCl in 0.03 M HCl. The absorbances at 257 and 249 nm were measured in order to estimate the content of adenylic and inosinic acids, respectively.

Content of H_2O_2

A highly sensitive colorimetric method based on the oxidation of phenolphthaline to phenolphthaleine in alkaline medium[12] was used.

Content of protein

Protein content was estimated by the method of Lowry *et al.*[13] using crystalline bovine serum albumin (Koch–Light) as a standard.

Content of SH groups

The content of SH groups was measured colorimetrically with 5,5′-dithiobis(2-nitrobenzoic acid) as described by Ellman[14]. The spectrophotometric procedure of Boyer[15] with *p*-chloromercuribenzoate was employed in our previous work[5] as an independent technique for the estimation of SH groups. The results obtained by means of both methods were identical in all the experiments.

RESULTS

Induced appearance of AMP-deaminating activity in highly purified preparations of monoamine oxidase

Effect of oxidizing reagents. A decrease in the tyramine- and appearance of histamine-deaminating activity, which were observed in highly purified bovine liver mitochondrial monoamine oxidase preparations treated with oxidizing reagents (*cf.* ref. 3), were accompanied by the appearance of high AMP-deaminating activity (Table I). The latter phenomenon was observed also in samples which did not possess a histamine-deaminating activity (Table I; experiments with oxidized glutathione).

Reversibility of the effect of oxidizing reagents. Treatment with reducing reagents

TABLE I

EFFECT OF OXIDIZING REAGENTS ON THE DEAMINATION OF NITROGENOUS COMPOUNDS BY AND CONTENT OF SH GROUPS IN HIGHLY PURIFIED BOVINE LIVER MITOCHONDRIAL MONOAMINE OXIDASE

The enzyme, purified 250-fold, was treated with oxidized oleic acid as described previously[3]. Samples, containing oxidized glutathione (10 mM), 0.2 mg of protein and 0.1 M citrate buffer (pH 6.7) to a total volume 2 ml, were kept at room temperature for 30 min and then dialyzed against the same buffer. Samples with H_2O_2 (0.1 mM) contained 1 mg of protein and 0.1 M citrate buffer (pH 6.7) in a total volume of 5 ml. The samples were kept at room temperature for 20 min, after which 250 μg of catalase were added and the samples were kept at the same temperature for 15 min. Optimal final concentrations (mM) of tyramine, histamine and AMP in the samples were 3.2, 25 and 10, respectively. Average values (from the results of 4–6 assays) ± standard error show the rate of deamination in nmoles of ammonia liberated per mg of protein per min and the content of SH groups in moles per 10^5 g of protein.

Treatment of enzyme	*Deamination (nmoles NH_3 per mg protein per min) of*			*SH groups*
	Tyramine	*Histamine*	*AMP*	
—	1141 ± 23	0	0	8
Oxidized oleic acid	120 ± 12	89 ± 4	118 ± 3	3
Oxidized glutathione	536 ± 31	0	129 ± 5	4
H_2O_2	412 ± 7	42 ± 4	73 ± 4	4

TABLE II

REVERSIBILITY OF THE QUALITATIVE ALTERATION IN CATALYTIC PROPERTIES OF BOVINE LIVER MITOCHONDRIAL MONOAMINE OXIDASE

Samples contained 0.2 mg of protein of the enzyme purified 250-fold. Experimental conditions have been described previously[3]. Concentrations of the substrates are as in Table I. Average values from the results of 4–6 assays.

Treatment of enzyme	*Deamination (nmoles NH_3 per mg protein per min) of*		*SH groups*
	Tyramine	*AMP*	
—	1141 ± 23	0	8
Oxidized oleic acid	120 ± 12	118 ± 3	3
Oxidized oleic acid, then $NaBH_4$	816 ± 10	0	6
Oxidized oleic acid, then Na_3AsO_3	918 ± 7	0	6
Oxidized oleic acid, then H_2S	983 ± 10	0	5
H_2O_2	412 ± 7	73 ± 4	4
H_2O_2, then $NaBH_4$	900 ± 17	0	—
H_2O_2, then H_2S	929 ± 8	0	—

(sodium borohydride, H_2S, sodium arsenite) of the monoamine oxidase preparations, which were preincubated with oxidized oleic acid or H_2O_2, completely inhibited the AMP-deaminating activity and partially restored up to the initial level the tyramine-deaminating activity and content of SH groups in the enzyme preparation (Table II).

In our previous experiments[3] treatment with reducing reagents of highly purified bovine liver mitochondrial monoamine oxidase, which had been preincubated with oxidized oleic acid, completely inhibited the induced histamine-deaminating activity.

Obligatory conditions for inducing AMP-deaminating activity in monoamine oxidases

Catalytic sites of monoamine oxidases. If the enzymatic activity of highly purified bovine liver mitochondrial monoamine oxidase was blocked by one of the irreversible monoamine oxidase inhibitors *e.g.* pargyline (*N*-methyl-*N*-benzyl-propynylamine·HCl) or tranylcypromine (*trans*-2-phenylcyclopropylamine·1/2 H_2SO_4), then the treatment with oxidized oleic acid of these enzyme preparations did not induce any AMP-deaminating activity. Treatment with oxidized oleic acid of those mitochondrial protein fractions, which did not possess monoamine oxidase activity[3], was not accompanied by the appearance of AMP-deaminating activity. These data suggest that the appearance of AMP-deaminating activity in monoamine oxidase as a result of treatment with oxidizing reagents requires the presence of accessable catalytic sites.

Titratable SH groups. Addition to highly purified bovine liver mitochondrial monoamine oxidase of *N*-ethylmaleimide, to a final of concentration of 10 mM, decreased the content of free SH groups (titratable with 5,5'-dithio-bis(2-nitrobenzoic acid)) from 8 to 5 moles per 10^5 g of protein. However, subsequent treatment with oxidized oleic acid induced, in these enzyme preparations, the appearance of a distinct ability to deaminate AMP; as compared with control samples (without 10 mM *N*-ethyl-maleimide) the rate of AMP deamination was decreased only by about 17%. Pre-incubation of the monoamine oxidase preparation with 100 mM *N*-ethylmaleimide

TABLE III

EFFECT OF PREINCUBATION WITH *N*-ETHYLMALEIMIDE OF BOVINE LIVER MITOCHONDRIAL MONOAMINE OXIDASE ON THE APPEARANCE OF AMP-DEAMINATING ACTIVITY AFTER TREATMENT OF THE ENZYME WITH OXIDIZED OLEIC ACID

The enzyme, purified 230-fold, was preincubated with *N*-ethylmaleimide in 0.1 M potassium phosphate buffer (pH 6.9) at room temperature for 30 min after which aliquots (3 ml, 0.24 mg of protein) were taken for the estimation of SH groups[14] and for treatment with oxidized oleic acid[3]. Average values from the results of 4–6 assays.

Concn of N-ethylmaleimide (mM)	*SH groups (moles per 10 g protein)*	*AMP deamination (nmoles/mg protein per min)*
0	8	118 ± 3
0.1	7	118 ± 3
1	7	100 ± 5
10	5	98 ± 6
100	3	0

decreased the content of SH groups to 3 moles per 10^5 g of protein; treatment with oxidized oleic acid of this enzyme preparation did not induce AMP-deaminating activity (Table III).

These data suggest (but do not prove) that alkylation of five SH groups (out of eight present per 10^5 g of protein) in the monoamine oxidase prevented the transformation of the enzyme with the appearance of AMP-deaminating activity, but alkylation of three SH groups did not influence this phenomenon. Therefore, it seems possible that, besides the functional integrity of the catalytic sites of monoamine oxidases, the presence of free SH groups, which are probably located away from the active sites of mitochondrial monoamine oxidases[16–18], may also be considered as an obligatory precondition for the transformation of monoamine oxidase.

Properties of the AMP-deaminating activity induced in the highly purified monoamine oxidase

Thermolability. Incubation for 5 min at 50 °C of the highly purified bovine liver mitochondrial monoamine oxidase, pretreated with oxidized oleic acid, inhibited deamination of AMP (which was assayed by two independent methods) by about 60% (Fig. 1).

Kinetics. For 30 min (at least) at 37 °C the liberation of ammonia in samples which contained highly purified bovine liver mitochondrial monoamine oxidase, pretreated with oxidized oleic acid and AMP, was directly proportional to the duration of incubation. Out of 200 nmoles of AMP added into the samples, about 78% were deaminated within 30 min of incubation.

Substrate and enzyme concentration. The highest rate of ammonia liberation was observed in samples containing monoamine oxidase, pretreated with oxidized oleic acid, and 10 mM AMP; an excess of AMP caused inhibition of the AMP-deaminating activity (Fig. 2). In the presence of the optimal concentration of AMP, addition to the samples of increasing amounts of the enzyme pretreated with oxidized oleic acid, was accompanied by a linear increase in the amount of ammonia liberated.

pH optimum. The highest rate of AMP deamination by bovine liver mitochondrial monoamine oxidase, which was pretreated with oxidized oleic acid, was

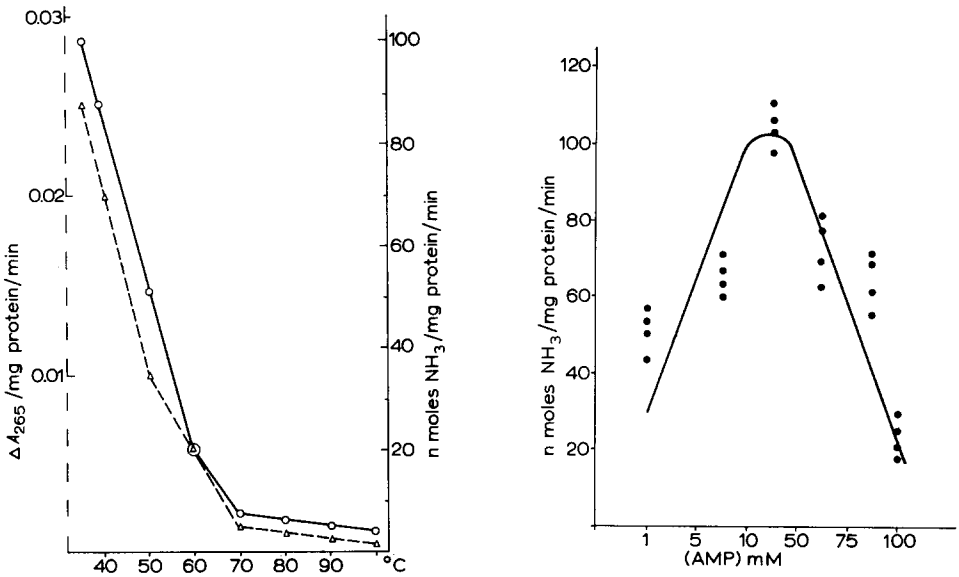

Fig. 1. Effect of controlled heating on deamination of AMP by bovine liver mitochondrial monoamine oxidase pretreated with oxidized oleic acid. Samples (total volume 2 ml) containing 0.12 mg of protein of the enzyme preparation, purified 250-fold compared to the homogenate, and pretreated with oxidized oleic acid (*cf.* ref. 3) in 0.1 M potassium phosphate buffer (pH 6.9) were kept for 5 min at the temperatures indicated on abscissa. The AMP-deaminating activity was then measured either spectrophotometrically (– – –) or by following the liberation of ammonia (———). Each point shows the result of a single assay.

Fig. 2. Effect of substrate concentration on the rate of AMP deamination by bovine liver mitochondrial monoamine oxidase pretreated with oxidized oleic acid.

observed (irrespective of the concentration of AMP in samples or of the method used for estimation of AMP-deaminating activity) at pH 6.9–7.0 (Fig. 3).

Stoichiometry. During incubation of AMP with highly purified bovine liver mitochondrial monoamine oxidase, pretreated with oxidized oleic acid, the formation of 1 mole of inosinic acid per mole of liberated ammonia was observed, but formation of H_2O_2 did not take place. These data suggest that in our experiments AMP was deaminated *via* a hydrolytic reaction[19].

Mixed substrates. Highly purified preparations of bovine liver mitochondrial monoamine oxidase, which were pretreated with oxidized oleic acid, deaminated (besides AMP) ADP and ATP (Table IV), but did not deaminate adenosine, adenine, adenosine 2′(3′)-monophosphate, guanosine 5′-monophosphate, NAD^+, NADH or FAD. Addition of both AMP and ATP (in optimal concentrations, found in special experiments) to the same samples which were then incubated with the enzyme preparation, lead to a considerable decrease (by about 40%) in the liberation of ammonia, compared to the corresponding values for deamination of each of the nucleotides (Tables IV). This phenomenon was not observed in the course of simultaneous incubation of AMP+histamine or AMP+tyramine. In these experiments the determined values of ammonia liberation were identical to or slightly (by about 15%) exceeded the calculated sum of the deamination rates of each of the nitrogenous compounds (Table IV). The data obtained suggest that deamination of various nucleotides in the course of their incubation with highly purified bovine liver mito-

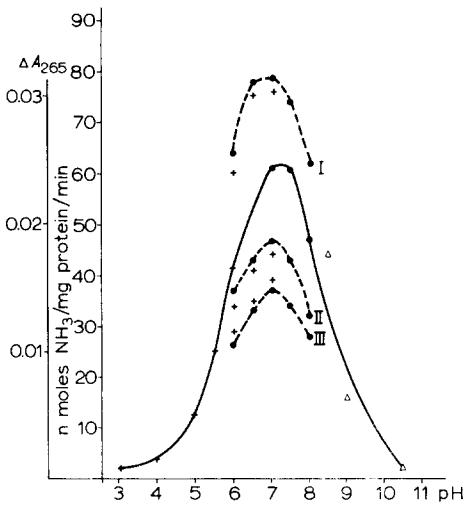

Fig. 3. Effect of pH on the rate of deamination of AMP by bovine liver mitochondrial monoamine oxidase pretreated with oxidized oleic acid. The enzyme preparation was purified 230-fold. When the reaction rate was estimated by measuring the liberation of ammonia (— — —), samples (total volume 2 ml) contained 0.16 mg of protein of the enzyme preparation. Final AMP concentrations (mM) were: (I) 10; (II) 5; (III) 50. The samples were incubated for 45 min. When the reaction rate was estimated by measuring the decrease in content of AMP (———) samples (total volume 3 ml) contained 0.06 mg of protein of the enzyme preparation. Concentration of AMP was 1.5 mM, incubation for 10 min. The pH values, indicated on the abscissa, were determined in parallel experiments by direct potentiometric measurements. ×—×, 0.2 M sodium acetate buffer; ●—●, 0.2 M potassium phosphate buffer; △—△, 0.2 M Tris–HCl buffer. Average values from the results of 4 assays are presented.

TABLE IV

EFFECT OF AMINES AND NUCLEOTIDES ON AMP DEAMINATION BY HIGHLY PURIFIED BOVINE LIVER MITOCHONDRIAL MONOAMINE OXIDASE PRETREATED WITH OXIDIZED OLEIC ACID

Average values (± standard error) from the results of 3–8 assays. The enzyme was purified 200-fold. Samples contained (in a total volume 2 ml) 0.2 mg of protein of the enzyme preparation, pretreated with oxidized oleic acid, substrates, and 0.1 M potassium phosphate buffer (pH 6.9).

Substrates	*Optimal concentrations (mM)*	*Liberation of ammonia (nmoles/mg protein per min)*
AMP	10	93 ± 2
ATP	10	20 ± 2
AMP + ATP	10 + 10	70 ± 2
ADP	2.5	42 ± 3
Histamine	25	94 ± 7
AMP + histamine	10 + 25	217 ± 4
Tyramine	3.2	141 ± 12
AMP + tyramine	10 + 3.2	230 ± 4

chondrial monoamine oxidase, pretreated with oxidized oleic acid, was catalyzed by a single active site which differed from the catalytic center involved in deamination of biogenic amines by these enzyme preparations.

Inhibitors. Pargyline or tranylcypromine did not influence the rate of deamination of AMP either by a preparation of highly purified bovine liver mitochondrial

TABLE V

EFFECT OF INHIBITORS ON ENZYMATIC DEAMINATION OF AMP

The enzyme was purified 200-fold. Samples (final volume 2 ml) contained 0.09 mg of protein of the enzyme preparation, one of the inhibitors and 0.1 M potassium phosphate buffer (pH 6.9). After preincubation for 30 min at room temperature, AMP (10 mM) was added and the rate of its deamination was estimated by measuring the liberation of ammonia. In control samples (without the inhibitors) 109 ± 3 nmoles of ammonia per mg protein per min were liberated. In experiments with AMP deaminase from rabbit skeletal muscles the samples contained 0.29 mg of protein of the enyzme preparation, 0.1 M citrate buffer (pH 6.5) in a total volume of 2 ml. In control samples (without the inhibitors) 157 ± 7 nmoles of ammonia per mg protein per min were liberated. Average values from the results of 3–5 assays show the inhibition (%) of the rate of AMP deamination.

Inhibitors	*Concn (mM)*	*Inhibition (%)*	
		Modified monoamine oxidase	*AMP deaminase from muscle*
Pargyline	0.1	14	9
Tranylcypromine	0.001	16	3
p-Chloromercuribenzoate	0.01	56	39
N-Ethylmaleimide	0.1	53	48
Iodoacetamide	0.01	55	22
Hydroxylamine	0.1	73	5
Isoniazid	0.1	81	11

monoamine oxidase, which was pretreated with oxidized oleic acid, or by a preparation of AMP deaminase from rabbit skeletal muscles (Table V). The activity of both enzyme preparations was inhibited by reagents, which react with SH groups (*p*-chloromercuribenzoate, *N*-ethylmaleimide, iodoacetamide). Carbonyl reagents—hydroxylamine and isoniazid (hydrazid of isonicotinic acid)—inhibited the deamination of AMP catalyzed only by the modified monoamine oxidase but not by AMP deaminase from rabbit skeletal muscles (Table V). Identical results were obtained in similar experiments with the estimation of AMP-deaminating activity by a spectrophotometric procedure[11].

Deamination of AMP (but not of histamine) by highly purified bovine liver mitochondrial monoamine oxidase, pretreated with oxidized oleic acid, was inhibited by the structural analogues of AMP adenosine 2′(3′)-monophosphate or 8-bromoadenosine 5′-monophosphate (Fig. 4). Dialysis (against 4 mM potassium phosphate buffer (pH 6.9) for 6 h with constant stirring) of the enzyme preparations pretreated with these inhibitors restored the AMP-deaminating activity to the initial level.

Thus, both adenosine 2′(3′)-monophosphate and 8-bromoadenosine 5′-monophosphate were reversible and selective inhibitors of deamination of AMP by highly purified preparations of bovine liver mitochondrial monoamine oxidase pretreated with oxidized oleic acid. Similar results were obtained in studies of the inhibitory effect of adenosine 2′(3′)-monophosphate on deamination of AMP by bovine liver mitochondrial membranes pretreated with oxidized oleic acid[20].

Experiments in vivo

Injections of oxidized oleic acid. Administration to rats of oxidized oleic acid under conditions such that it caused distinct radiomimetic effects[9] was accompanied

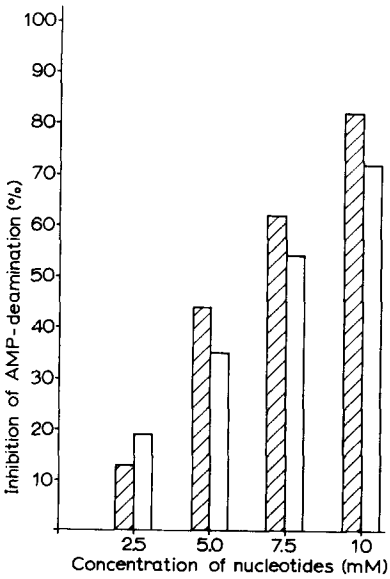

Fig. 4. Effect of adenosine 2′(3′)-monophosphate (shaded bars) and 8-bromoadenosine-5′-monophosphate (unshaded bars) on AMP-deaminating activity of highly purified bovine liver mitochondrial monoamine oxidase, pretreated with oxidized oleic acid. The enzyme was purified 200-fold. The samples (total volume 2 ml) contained 0.3 mg of protein of the enzyme preparation in 0.1 M potassium phosphate buffer (pH 6.9). The structural analogues of AMP were preincubated with the enzyme preparation for 30 min at room temperature after which AMP (10 mM) was added to the samples and the AMP-deaminating activity was estimated by measuring the rates of ammonia liberation. In control samples (which did not contain the inhibitors) the mean value of ammonia liberation was 92 ± 4 nmoles per mg of protein per min. Average values from the results of 3–5 assays are presented.

TABLE VI

EFFECT OF ADMINISTRATION TO RATS OF IPRONIAZID OR OXIDIZED OLEIC ACID ON DEAMINATION OF SOME NITROGENOUS COMPOUNDS BY RAT LIVER MITOCHONDRIA

Gradually increasing amounts (from 0.04 to 0.1 ml) of oxidized oleic acid were injected intraperitoneally to rats (number of which is shown in parenthesis) daily for 5 days. Iproniazid (150 mg/kg body weight) was injected intraperitoneally twice:16 h before administration of oxidized oleic acid and then 72 h after the first injection of the inhibitor. The animals were sacrificed by decapitation and mitochondria were isolated[21] from their liver. Samples (total volume 2 ml) contained 1.5–4 mg of protein of the mitochondria, one of the substrates in optimal concentrations (mM): serotonin 5, tyramine 3.2, cadaverine 10, AMP 10, and 0.1 M potassium phosphate buffer (pH 7.4). Statistically significant differences are shown compared to the control.

Treatment of animals	*Deamination (nmoles NH_3 per mg protein per min) of*			
	Serotonin	*Tyramine*	*Cadaverine*	*AMP*
0.9% NaCl (control)	8 ± 0.1	18.4 ± 0.2 (24)	0 (24)	2.5 ± 0.3 (10)
Oxidized oleic acid	5.5 ± 0.1* (20)	11.2 ± 1.3** (20)	1.6 ± 0.2 (16)	8.6 ± 0.7** (6)
Iproniazid	0.4 ± 0.06*** (14)	—	—	—
Iproniazid + oxidized oleic acid	0.8 ± 0.4*** (6)	0 (6)	0 (6)	0 (6)

* $P < 0.03$.
** $P < 0.01$.
*** $P < 0.001$.

by a decrease in monoamine oxidase (substrates serotonin or tyramine) activity; the appearance of cadaverine-deaminating activity and a significant (about 3.5-fold) increase in AMP-deaminating activity in liver mitochondria (Table VI). This phenomenon did not take place in animals in which before the administration of oxidized oleic acid the monoamine oxidase activity was blocked by iproniazid. These data are in agreement with the results of experiments carried out *in vitro* and suggest that the presence of the intact catalytic site in mitochondrial monoamine oidases is one of the obligatory preconditions for the transformation of their enzymatic properties[3,7,10].

TABLE VII

EFFECT OF IRRADIATION (700 rad) AND ADMINISTRATION TO RATS OF ISONIAZID OR MONOAMINE OXIDASE INHIBITORS ON DEAMINATION OF SOME NITROGENOUS COMPOUNDS BY RAT LIVER MITOCHONDRIA

Rats were sacrificed within 7 days after a single[9] X-ray irradiation (700 rad). Mitochondria were isolated from liver tissue and the rates of deamination of nitrogenous compounds were estimated (final concentrations of histamine and putrescine in samples were 10 mM, of the other substrates, see Table VI). Isoniazid (75 mg/kg body weight in 0.5 ml of 0.9% NaCl) was injected intraperitoneally (twice in one day) at the 7th day after irradiation. Pargyline (110 mg/kg of body weight) was injected intraperitoneally 16 h before irradiation and then the injections were repeated every 40 h. Iproniazid administration as in Table VI. Statistically significant differences are shown compared to the control. Average values from the results of 4–7 assays are presented.

Treatment of animals	*Deamination (nmoles NH_3 per mg protein per min) of*				
	Tyramine	*Serotonin*	*Histamine*	*Putrescine*	*AMP*
0.9% NaCl (control)	32 ± 1	15 ± 1.5	0	0	6.3 ± 0.2
Irradiation +0.9% NaCl	23 ± 3*	12 ± 3	1 ± 0.4	1.5 ± 0.4	14.3 ± 3***
Irradiation +isoniazid	19.4 ± 1***	9.4 ± 1**	1 ± 0.2	1 ± 0.1	6 ± 1
Isoniazid	31 ± 1.6	12 ± 0.5	—	—	—
Iproniazid +irradiation	—	0.2 ± 0.1***	0	0	0
Pargyline +irradiation	1.5 ± 0.1***	2.5 ± 0.3***	0	0	1.9 ± 0.1***

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Experimental radiation injury. In mitochondria isolated from the liver of irradiated rats, there was a significant (about 2.3-fold) increase in AMP-deaminating activity (Table VII), besides the decrease in monoamine oxidase activity and appearance of an ability to deaminate substrates of diamine oxidase (histamine or putrescine) (*cf.* ref. 9). Injections into the irradiated rats of isoniazid, which *in vitro* inhibited the AMP-deaminating activity of monoamine oxidase pretreated with oxidized oleic acid, decreased the rate of AMP deamination in liver mitochondria of irradiated rats to the level characteristic for control rats. Administration of monoamine oxidase inhibitors before the irradiation either completely (iproniazid) or partially (pargyline) prevented the increase in AMP-deaminating activity in irradiated rats (Table VII).

TABLE VIII

IMPAIRMENTS OF DEAMINATION OF SOME NITROGENOUS COMPOUNDS BY RAT LIVER MITOCHONDRIAL MEMBRANES IN HYPERVITAMINOSIS D_2

Dispersions of vitamin D_2 were injected into rats as previously described[8] for 6 days. Mitochondrial fractions[22] isolated from liver of experimental and control animals were subjected to repeated freezing and thawing with subsequent washing of the mitochondrial membranes with 0.01 M potassium phosphate buffer (pH 7.4). The preparation of mitochondrial membranes thus obtained was kept at −20 °C for several weeks. Samples (final volume 2 ml) contained 3–4 mg of protein of the mitochondrial membranes, one of the substrates in optimal concentrations (mM): tyramine 3.2, histamine or AMP 10, and 0.2 M potassium phosphate buffer (pH 7.4).

Treatment of animals	*Deamination (nmoles NH_3 per mg protein per min) of*		
	Tyramine	*Histamine*	*AMP*
0.9% NaCl (control)	19 ± 0.4 (16)	0 (6)	0 (7)
Vitamin D_2	11 ± 0.7 (16)	1.5 ± 0.3 (4)	4.5 ± 1 (5)

Hypervitaminosis D_2. Data presented in Table VIII, show that the hypervitaminosis D_2 was accompanied not only by a decrease in monoamine oxidase activity and appearance of an ability to deaminate histamine (*cf.* ref. 8), but also by an induction of AMP-deaminating activity in mitochondrial membranes from rat liver.

These data suggest that not only the irradiation injury (or administration of a radiomimetic compound oxidized oleic acid) but also a quite different pathological state (hypervitaminosis D_2) which is characterized by accumulation of lipid peroxides in tissues[8], was accompanied by transformation of catalytic properties of monoamine oxidases.

DISCUSSION

Treatment with some oxidizing reagents (including oxidized oleic acid which occurs in animal tissues) of highly purified preparations of an oxidative enzyme, bovine liver mitochondrial monoamine oxidase, was accompanied by the appearance of an AMP-deaminating activity. Rat liver mitochondrial monoamine oxidase[7] or soluble bacterial monoamine oxidase from *Sarcina lutea*[5] behave similarly on treatment with oxidizing reagents.

In monoamine oxidases, treated with oxidizing reagents, oxidation of a part of the SH groups was observed[3,5,7]. However, oxidation of certain SH groups in the monoamine oxidase may not be considered as a single possible cause of inducing in these enzyme preparations of an AMP-deaminating activity. This phenomenon was observed[23] in the course of treatment of highly purified bovine liver mitochondrial monoamine oxidase (or of the mitochondrial membranes) not only with oxidized oleic acid but also with other uncoupling reagents (for example, 2,4-dinitrophenol) which did not influence the content of free SH groups in samples.

The properties of naturally occurring AMP deaminases[24–26] significantly differ from those of the AMP-deaminating activity induced in highly purified preparations

of bovine liver mitochondrial monoamine oxidase. Thus, AMP deaminases from rat liver[27], some plants[28] or *Azotobacter vinelandii*[29] deaminate only AMP but not ADP or ATP. The latter nucleotides activated deamination of AMP by enzyme preparations isolated from liver[27] or brain[30] tissues. Highly purified preparations of bovine liver mitochondrial monoamine oxidase after treatment with oxidized oleic acid deaminated not only AMP but also ADP and ATP (albeit at a lower rate); addition of ATP to the samples, which contained AMP, inhibited deamination of the latter. Although the carbonyl reagents (isoniazid, hydroxylamine) did not influence the deamination of AMP by AMP deaminase from rabbit skeletal muscles, these reagents inhibited the AMP-deaminating activity of highly purified bovine liver mitochondrial monoamine oxidase pretreated with oxidized oleic acid.

Our data suggest that the ability of mitochondrial monoamine oxidases to undergo transformation of their catalytic properties, accompanied by appearance of AMP-deaminating activity, may be realized *in vivo* (*cf.* ref. 31). Thus, this phenomenon may occur under pathological conditions (radiation injury, hypervitaminosis D_2) characterized by accumulation in tissues of lipid peroxides, resembling, in chemical properties and biological effects, the oxidized oleic acid[32]. Similar findings were made in mitochondria and nuclei of tumor-bearing mice liver[33] (where there was a considerable increase in content of lipid peroxides in the course of development of ascites carcinoma[34]), or in rat liver mitochondria under conditions of decrease in antioxidative properties of tissues due to K-hypovitaminosis[35].

The appearance of (or significant increase in) AMP-deaminating activity in cellular structures may be accompanied by deamination of some nucleotide coenzymes[20]. Thus, there was an increase in tissue concentration of inosinic acid in irradiation injury[36]. Increase in deamination of AMP and of other nucleotides may be pathogenetically important. The following data support this assumption: (1) in the course of development of irradiation injury there was a sharp increase in AMP-deaminating activity in rat liver mitochondria[37]; (2) in radio-resistant animal species the increase in AMP-deaminating activity in liver or intestinal mitochondrial fractions was much less then in radio-sensitive species[38]; (3) inhibition by the structural analogues of AMP adenosine 3'-monophosphate or adenosine 2'(3')-monophosphate (*i.e.* by competive inhibitors of the AMP-deaminating activity[20]) of the reaction of AMP deamination *in vivo* in pathological states, which were characterized by accumulation of lipid peroxides in tissues, considerably improved the general state of experimental animals and increased their survival from radiation injury[37] and Ehrlich ascites carcinoma[33].

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