DISTURBANCES OF DEAMINATION OF SOME NITROGEN COMPOUNDS AND THEIR EXPERIMENTAL NORMALIZATION

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A decrease in monoamine oxidase activity accompanied by the appearance of histamine- and AMP-deaminase activities was found in the liver mitochondria of rats after x-ray irradiation or intraperitoneal injection of oxidized oleic acid. Repeated parenternal injections of adenosine-2'(3')-monophosphate into the animals normalizes these disturbances of deamination of nitrogen compounds.

If liver mitochondria are treated with oxidized oleic acid, their monoamine oxidase activity is reversibly depressed; this is accompanied by uncharacteristic ability of the mitochondria to deaminate histamine and other nitrogen compounds, especially adenosine-5'-monophosphate (AMP) [4]. Under these conditions not only is adenosine-2'(3')-monophosphate not deaminated, but it inhibits the deamination of AMP [4].

In the investigation described below, disturbances of the deamination of serotonin and other nitrogen compounds in rat liver mitochondria were investigated in an experimental pathological state (radiation sickness), accompanied by the accumulation of lipid peroxides, similar in some of their properties to oxidized oleic acid [8], in the tissues. An attempt was also made to normalize the disturbances found by repeated parenteral injections of adenosine-2'(3')-monophosphate into the animals.

EXPERIMENTAL METHOD

Male albino rats weighing 120-150 g were used in the experiments. The animals were irradiated with x-rays on a type RUM-11 apparatus (dose rate 32 rad/min, voltage 180 kV, current 15 mA, filters: 0.5 mm copper and 1 mm aluminum). The methods of preparing the oxidized oleic acid and of its administration to the animals, and also the methods of isolating mitochondria, determining protein, and measuring the rate of deamination, have been described previously [3].

EXPERIMENTAL RESULTS AND DISCUSSION

On the 4th day after irradiation (1,000 rad) of the rats, the rate of deamination of serotonin in the liver mitochondria was reduced and the mitochondria were able to deaminate histamine or AMP (Table 1). Similar disturbances of the deamination of nitrogen compounds were found in the liver mitochondria of rats receiving repeated intraperitoneal injections of the radiomimetic substance oxidized oleic acid (1 mmole O_2/g of the substance, based on the results of determination of the hydroperoxide content by an iodometric method [9]) by a scheme developed and described previously [3], in doses equivalent in resulting mortality to the dose of x-ray irradiation used [3].

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TABLE 1. Disturbances of Deamination of Nitrogen Compound in Liver Mitochondria of Rats after X-Ray Irradiation or Administration of Oxidized Oleic Acid and Normalizing Effect of Adenosine-2'(3')-Monophosphate (M ± m)

Procedure	Rate of deamination (in nmoles NH_3/mg protein/min)		
	serotonin	histamine	AMP
Control	10.7 ± 1.2 (8)	0 (6)	0 (6)
Irradiation	7.2 ± 1.0 (6)	0.4 ± 0.04 (6)	2.5 ± 0.1 (6)
Irradiation followed by adenosine-2'(3')-monophosphate	7.9 ± 0.1 (6)	0 (6)	0.2 ± 0.03 (6)
Irradiation followed by AMP	7.9 ± 1.1 (4)	1.1 ± 0.04 (4)	2.5 ± 0.4 (4)
Oxidized oleic acid	7.0 ± 1.4 (6)	1.0 ± 0.06 (4)	2.2 ± 0.2 (4)
Oxidized oleic acid followed by adenosine-2'(3')-monophosphate	9.7 ± 0.3 (4)	0 (4)	0.1 ± 0.08 (4)

Note. Each sample contained 3-5 mg mitochondrial protein, one of the substrates in the following optimal ("saturating") concentrations (in mM): serotonin 5, histamine 10, AMP 5, and also 0.1 M K–phosphate buffer, pH 7.4, to a total volume of 1.8 ml. For a description of the technique of the determinations, see [1, 3]. Number of experiments in parenthesis.

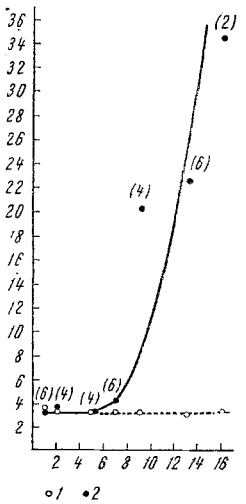

Fig. 1. Changes in rate of deamination of AMP in rat liver mitochondria during development of radiation sickness. Each point shows the result of one determination. Number of experiments in parenthesis. 1) Control; 2) radiation sickness. Abscissa, time after irradiation of rats (in days); ordinate, rate of deamination of AMP (in nmoles liberated ammonia/mg protein/min).

Adenosine-2'(3')-monophosphate (sodium salt) was injected intraperitoneally (60 mg/100 g body weight) 6 h after irradiation (or after the first injection of oxidized oleic acid), and thereafter every 8 h for 3 days. The control animals received intraperitoneal injections of equal volumes of physiological saline. The dose and scheme of administration of adenosine-2'(3')-monophosphate were chosen so as to maintain a concentration of free nucleotides of the order of 1 mM in the liver tissue (the nucleotide content was determined quantitatively by thin-layer chromatography on DEAE-cellulose with the kind co-operation of V. L. Kozel'tsev and L. B. Rebrov). In the concentration specified, adenosine-2'(3')-monophosphate inhibits deamination of AMP by rat liver mitochondria treated in vitro with oxidized oleic acid [4]. As the results in Table 1 show, administration of adenosine-2'(3')-monophosphate (but not adenosine-5'-monophosphate) to rats after irradiation or treatment with oxidized oleic acid not only virtually completely abolishes the AMP- and histamine-deaminase activity appearing in the liver mitochondria under these conditions, but also facilitates the normalization of monoamine oxidase activity. The action of adenosine-2'(3')-monophosphate which cannot be replaced under these conditions by a AMP cannot be attributed to a nonspecific effect (administration of an organic compound to the irradiated animals). The results accord with the views, based on the study of reversible qualitative changes in catalytic properties of highly-purified preparations of monoamine oxidases [2, 5, 11], that reversible qualitative changes in the catalytic properties of monoamine oxidases, with the appearance of the normally absent ability to deaminate histamine, AMP (and many nucleotide coenzymes), and other nitrogen compounds [7], can take place in pathological states accompanied by the accumulation of products of peroxide oxidation of lipids in the tissues [1, 3, 6].

As the radiation sickness developed progressively the intensity of these disturbances of deamination of the nitrogen compounds increased [6]. The sharp increase in AMP-deaminase activity in the rat liver mitochondria after a single dose of whole-body γ-ray irradiation on a GUBÉ-800 apparatus (dose rate 50 rad/min) in a dose of 800 rad is shown in Fig. 1. An increase in the inosinic acid concentration in the tissues in radiation sickness has been reported previously [10].

The results showed that normalization of the disturbances of deamination of nitrogen compounds described above as the result of administration of adenosine-2'(3')-monophosphate was accompanied by a decrease in the mortality of the rats after a single whole-body irradiation with x-rays (650 rad). For instance, in the group of animals receiving adenosine-2'(3')-monophosphate after irradiation, nine (of the ten) animals still survived on the 10th day, whereas in the first control group (irradiation followed by injection of physiological saline) only four of the ten animals survived; in the second control group (irradiation followed by administration of AMP) only three of the ten survived. These observations are evidence of the possible pathogenetic role of the disturbances of deamination of nitrogen compounds studied in this investigation in experimental pathological states.

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