STIMULATION OF ADENYLATE DEAMINASE ACTIVITY BY UNCOUPLING AGENTS

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Uncoupling agents with different chemical structure, such as oxidized oleic acid, cause the appearance of adenylate deaminase activity in highly purified preparations of monoamine oxidase (MAO) or in mitochondrial membranes of bovine liver. Some connection is considered to exist between the appearance of (or a sharp rise in) adenylate deaminase activity (probably resulting from modification of the properties of mitochondrial MAO) and the uncoupling action.

The treatment of highly purified monoamine oxidases (MAO) with certain oxidizing agents (including oxidized oleic acid) causes the appearance of adenylate deaminase activity in these enzyme preparations [1, 10, 11]. This effect has also been observed in experiments on mitochondria [4] and in the intact organism [5].

Oleic acid and other unsaturated fatty acids, which are readily oxidized by peroxides, are uncouplers of tissue respiration and phosphorylation [7]. On the basis of the views [2, 7] that the amino group of the adenyl residue participates in the binding of inorganic phosphate during the formation of high-energy phosphate bonds it could be assumed that the uncoupling effect of oleic acid and its ability to increase the adenylate deaminase activity of the mitochondria are interconnected.

To test this hypothesis the action of uncoupling compounds of different chemical structure could be studied on the adenylate deaminase activity of mitochondria and of highly purified preparations of mitochondrial MAO.

EXPERIMENTAL METHOD

The methods of isolation of the mitochondria [4] and of MAO preparations with a degree of purity 250 times greater than the homogenate [8] were described earlier [8]. In the process of isolation the mitochondria were frozen and thawed and the residue of mitochondrial membranes was washed with hypotonic buffer solution [4]. The content of peroxides in the preparations of oxidized oleic acid (OOA) was 1.3-1.5 mmole O_2/g fatty acid. The solutions of p-trifluoromethoxy-carbonyl-cyanidophenylhydrazone (FCCP), tetrachlorotrifluoromethylbenzimidazole (TFB) and also the 2,4-dinitrophenol (DNP), rotenone, p-di(2-chloroethylamine)phenylacetic (DCPA) and (2-chloroethylamine)phenylacetic (CPA) acids were kindly supplied by V. P. Skulachev. One of these uncoupling agents (or thyroxine "Reanal") was added to the sample containing either mitochondrial membranes (3-7 mg protein) or highly purified preparations of MAO from bovine liver (0.09-0.15 mg protein). The volume of the samples was adjusted to 1.8 ml either with 0.2 M phosphate buffer, pH 7.4 (in the experiments with mitochondria), or with 0.1 M citrate buffer, pH 6.7 (in the experiments with the purified enzyme). After preincubation for 20-30 min at room temperature, one of the nitrogen compounds was added to the sample in the following optimal ("saturating") final concentrations (in mmoles):

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TABLE 1. Effect of Some Oxidizing and Uncoupling Agents on Enzyme Activity and Content of Thiol Groups in Highly Purified MAO Preparations from Mitochondria of Bovine Liver (values of M ± m for results of 4-6 experiments)

Treatment of enzyme	Concentration (mM)	SH groups (moles/10 g protein)	Deamination (nmoles/mg protein per minute)		
			tyramine	histamine	AMP
—		8	1141±23	0	0
OOA	10,0	3	120±12	89±4	118±3
Oxidized glutathione	10,0	4	536±31	0	129±5
Hydrogen peroxide	0,1	4	412±7	42±4	73±4
DNP	0,1	7	201±7	31±2	70±3
Rotenone	0,01	—	383±10	0	80±7
DCPA	0,1	4	216±18	0	86±2
DCPA	0,1	—	1092±56	0	0

TABLE 2. Deamination of Some Nitrogen Compounds on Incubation with Preparations of Bovine Liver Mitochondrial Membranes Treated with Uncoupling Agents (values of M ± m for results of 4-6 experiments)

Reagents	Concentration (mM)	Deamination (nmoles/mg protein per minute		
		tyramine	histamine	AMP
—		13±1,2	0	0
OOA*	10,0	0,8±0,1	1,9±0,2	13±0,3
Vitamin D_2*	0,0025	—	2,0±0,05	4,5±1
DNP	0,1	0,9±0,1	1,2±0,5	3,7±0,1
Rotenone	0,1	2,7±0,3	0,8±0,2	4,0±0,2
DCPA*	1,0	1,2±1	0	7,4±0,9
CPA	1	12,1±1,3	0	0
Thyroxine	0,01	2,0±0,7	0	4,2±1,3
Amobarbital	0,01	10,2±0,2	0	0
FCCP	0,01	4,8±0,7	2,1±0,2	2,8±1,0
TFB	0,01	8,3±1,1	0,8±0,1	2,2±0,2

*Uncouplers whose action was tested in optimal concentrations (of 5-6 studied).

tyramine·HCl (3.2), cadaverine·2 HCl (10), histamine·2 HCl (10), AMP (5). The samples were incubated for 45 min at 37°C in an atmosphere of oxygen. The methods of fixation of the samples, of measuring the rate of deamination of the nitrogen compounds, and of determining the content and thiol groups were described before [4, 8].

EXPERIMENTAL RESULTS

Uncoupling agents with such widely different chemical structures as DNP, rotenone, and DCPA, in the concentrations usually used, like oxidizing agents [1], caused adenylate deaminase activity to appear in highly purified preparations of mitochondrial MAO from bovine liver (Table 1).

Deamination of adenylic acid led to the formation of inosinic acid, as was established by several independent methods. CPA, which is not an uncoupling agent [6], did not cause adenylate deaminase activity to appear (the rate of deamination of tyramine by the enzyme preparation was not reduced, unlike in the experiments with the uncouplers). The appearance of histamine deaminase activity and a decrease in the content of thiol groups did not always accompany the appearance of adenylate deaminase activity in the enzyme preparations treated with uncoupling agents. These results are evidence that the transformation of MAO, like that of other enzymes [3], may probably be based on processes of different chemical natures, and not only the oxidation of thiol groups [8].

The ability of uncouplers to stimulate adenylate deaminase activity is also exhibited in experiments on mitochondrial membranes from bovine liver (Table 2). In order of decreasing effectiveness as stimulators of adenylate deaminase activity the uncoupling agents can be arranged as follows: FCCP > DNP > DCPA > OOA.

This series is slightly reminiscent of the relationships found under totally different conditions (composition of the samples, duration of incubation, and so on) when the effects of uncouplers were studied on respiration of mitochondria and also on reconstructed complexes of respiratory enzymes and artificial phospholipid membranes [9]. These investigations, like those now described, showed that relatively low concentrations of uncouplers stimulate, while higher concentrations inhibit, the processes which were studied.

The results are in agreement with the view that uncouplers, like OOA, can stimulate adenylate deaminase activity in mitochondria (or in highly purified MAO preparations) by inducing a qualitative change (transformation) in the catalytic properties of the MAO. However, the chemical bases of the MAO transformation induced by OOA are probably totally different from those of the transformation induced by other uncouplers.

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