ROLE OF MITOCHONDRIAL PEROXIDATION OF LIPIDS IN THEIR HYDROLYSIS BY ENDOGENOUS PHOSPHOLIPASE A_2

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Among the natural factors which modify the lipid bilayer of mitochondrial membranes, one of special importance is the endogenous phospholipase A_2 of these organelles. This enzyme, located in the two mitochondrial membranes [3, 11], is involved in regulation of the fatty acid and phospholipid compositions of mitochondria, in acyl-transfer reactions, and in the control of the rate of renewal of mitochondrial lipids [8, 11]. Recently the attention of research workers has been concentrated on elucidation of the connection between the efficiency of lipid hydrolysis by membrane phospholipases and the intensity of another factor in lipid degradation, namely peroxidation [1, 2, 4, 6, 12]. In most cases the data obtained are evidence of the activating action of lipid peroxidation (LPO) on phospholipase [1, 4, 6, 12], but information of the opposite kind also is available [2]. Although it is not clear to what extent this contradiction is due to differences between the objects or experimental conditions used, the study of the role of LPO in hydrolysis of individual fractions of mitochondrial phospholipids by endogenous phospholipase A_2 is of interest on its own account.

The investigation described below was undertaken to study this problem.

EXPERIMENTAL METHOD

Mitochondria were isolated from the liver of Wistar rats weighing 200-250 g by differential centrifugation [9] in medium containing 250 mM sucrose, 5 mM Tris-buffer, pH 7.4 (4°C). Lipids were extracted from the mitochondria by the method in [7], and this was followed by chromatography and quantitative analysis of the material as in [5]. The intensity of mitochondrial LPO was estimated by the accumulation of malonic dialdehyde (MDA) [10]. The mitochondrial protein concentration was determined by the biuret reaction.

EXPERIMENTAL RESULTS

Auto-oxidation of the mitochondrial lipids differed from their peroxidation induced by pro-oxidants, not only in the quantity of MDA, but also in the kinetics of its accumulation (Fig. 1). In the first case accumulation of LPO products took place during incubation for 2 h at a relatively constant rate, whereas in induced LPO MDA accumulation was most rapid during the first hour of incubation.

The results of recording the lipid composition of mitochondria incubated under different conditions are given in Table 1. It follows from these data that the greatest quantitative changes (in absolute figures) took place in two fractions, namely phospholipids and FFA. Incidentally, the decrease in the relative content of the former in the mitochondria was accompanied by a rise in the level of the latter. This suggests that under these experimental conditions mitochondrial lipids are degraded mainly through their hydrolysis by phospholipase A_2. So far as the other fractions of the lipids studied are concerned, although the changes in their relative content appear considerable (the metFFA level was raised 2.1-2.3 times, esChL 2-2.5 times, and MG by 1.5-2.9 times, a fall in the concentrations of DG and TG by 1.4-1.5 times), all these fractions except TG were found in the mitochondria in low (virtually at the trace level) absolute quantities. This fact, together with the absence of data on the structural and functional role of these minor fractions of mitochondrial lipids, evidently makes it possible to omit the analysis of these changes.

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TABLE 1. Effect of Conditions of Incubation on Lipid Composition of Mitochondria (in % of total lipids; M ± m)

Fraction	Incubation time, min				
	0	30	60	90	120
TPL					
A	85,7±4,1	82,0±4,2	78,0±5,1	74,2±6,1	72,1±4,5*
B	—	82,9±3,1	75,7±4,3	72,5±5,2	69,4±4,4*
FFA					
A	4,9±0,5	7,3±0,5	14,0±1,3	18,1±1,6	20,6±2,0***
B	—	8,5±0,6	16,6±1,2	19,9±2,1	22,9±1,4***
metFFA					
A	0,3±0,01	0,39±0,04	0,54±0,05	0,65±0,1	0,7±0,05***
B	—	0,35±0,01	0,4±0,02	0,61±0,03	0,64±0,05***
ChL					
A	5,4±0,6	5,0±0,6	4,3±0,03	4,0±0,4	3,7±0,3*
B	—	4,0±0,1	3,9±0,2	3,5±0,2	3.4±0,1*
esChL					
A	0,2±0,01	0,25±0,01	0,28±0,02	0,32±0,01	0,5±0,03***
B	—	0,34±0,01	0,60±0,05	0,62±0,03	0,4±0,02***
MG					
A	0,3±0,05	0,40±0,01	0,40±0,01	0,53±0,02	0,9±0,03***
B	—	0,32±0,01	0,34±0,03	0,35±0,05	0.5±0,04*
DG					
A	0,46±0,02	0,40±0,01	0,38±0,02	0,35±0,04	0,32±0,02***
B	—	0,66±0,01	0,74±0,03	0,48±0,04	0,30±0,08
TG					
A	2,8±0,3	2,3±0,04	2,2±0,07	2,0±0,1	1,8±0,05**
B	—	2,7±0,04	2,4±0,09	2,2±0,1	2,0±0,08*

Legend. Here and in Table 2: data shown in the form of mean value and its error, determined from two series of experiments with 13-16 repetitions in each series. A) Auto-oxidation, B) oxidation in the presence of $FeSO_4$ (20 μM) and ascorbate (0.2 mM). TPL) Total phospholipid fraction; FFA and metFFA) free fatty acids and their methyl esters; ChL and esChL) cholesterol and its esters; MG, DG, TG) mono-, di-, and triglycerides respectively. Criterion of significance of differences compared with initial level: *p < 0.05, **p < 0.01, ***p < 0.001.

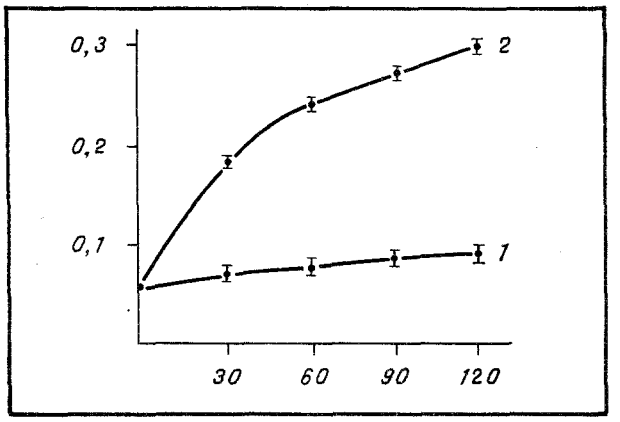

Fig. 1. Effect of LPO inducers on MDA accumulation in liver mitochondria. Abscissa, incubation time (in min); ordinate, optical density at 532 nm. 1) Control; 2) $FeSO_4$ (20 μM) + ascorbate (0.2 mM). Incubation medium (in mM): KCl 115, NaH_2PO_4 1, Tris-HCl 5, pH 7.4 (36°C). Protein concentration 1.5 mg/ml.

The results of a study of the time course of the content of individual phospholipid fractions of the two mitochondrial samples tested are given in Table 2. At a low level of LPO (sample A), it will be noted, hydrolysis of the three major phospholipid components of the mitochondria took place relatively uniformly. For instance, toward the end of the 2nd hour of incubation the concentrations of PC, PE, and CL had fallen by 18, 25, and 33% respectively compared with their initial level. A different picture was observed for mitochondria incubated with pro-oxidants (specimen B): the fall in the PC concentration toward

TABLE 2. Effect of Conditions of Incubation on Phospholipid Composition of Mitochondria (in % of total content of phosphatides; M ± m)

Fraction	Incubation time, min				
	0	30	60	90	120
PC					
A	34,1±1,4	32,9±1,3	32,0±1,4	30,0±1,5	28,0±1,2
B	—	35,6±1,4	33,9±1,5	33,6±1,5	32,7±1,5*
LPC					
A	1,6±0,2	1,8±0,2	2,0±0,2	2,2±0,3	2,4±0,2
B	—	2,7±0,4	2,4±0,4	4,3±0,6	3,3±0,6
PE					
A	26,0±1,1	25,5±1,1	24,5±1,4	22,0±1,3	19,6±1,4
B	—	14,7±1,7	13,4±1,0	9,9±1,0	9,5±0,9***
LPE					
A	10,6±0,9	9,5±0,9	9,0±0,7	9,3±0,9	8,9±0,9
B	—	8,4±0,7	6,7±0,6	6,0±0,5	6,3±0,5**
CL					
A	18,1±1,0	16,2±1,4	15,8±1,1	13,0±1,2	12,1±0,7
B	—	17,3±1,7	16,7±1,1	15,3±0,8	14,6±0,8**
LCL					
A	1,2±0,2	1,0±0,1	1,0±0,1	0,9±0,1	0,8±0,1
B	—	1,4±0,2	1,4±0,2	1,5±0,2	1,6±0,2**
PS					
A	1,8±0,5	2,0±0,4	2,1±0,3	2,4±0,3	2,5±0,4
B	—	2,4±0,5	2,7±0,4	4,8±0,5	2,6±0,4
PI					
A	2,1±0,5	2,0±0,5	2,2±0,5	2,2±0,4	2,5±0,4
B	—	2,2±0,2	2,4±0,3	2,5±0,2	2,6±0,3
PA					
A	1,4±0,4	2,5±0,2	3,0±0,3	4,1±0,4	5,4±0,8****
B	—	3,7±0,5	5,3±0,6	5,8±0,7	6,3±0,9****
LPA					
A	1,9±0,2	3,6±0,6	3,8±0,6	5,6±0,9	8,9±0,8****
B	—	5,5±0,4	6,5±0,4	7,6±0,5	8,9±0,6****
SM					
A	4,2±0,9	3,9±0,5	3,7±0,5	4,1±0,4	6,9±0,6
B	—	5,0±0,8	5,4±0,7	7,8±0,9	8,3±0,9

Legend. PC, PE, CL, PA) phosphatidylcholine, phosphatidylethanolamine, cardiolipin, and phosphatidic acid respectively; LPC, LPE, LCL, LPA) the lyso forms of these compounds respectively; PS) phosphatidylserine; PI) phosphatidylinositol; SM) sphingomyelin. Criterion of significance of differences between A and B: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Criterion of significance of difference compared with initial level for PA and LPA: ****$p < 0.001$.

the end of incubation was not significant (only 4%), CL fell by 19%, whereas the PE fraction fell by 63% compared with its initial level.

The increase in the PA concentration in the mitochondria toward the end of incubation is noteworthy (Table 2). This is all the more interesting because a more than threefold increase in the PA concentration took place in both specimens toward the end of incubation despite an almost fivefold increase in the fraction of its hydrolysis product — LPA. The possibility of PA synthesis under these experimental conditions can evidently be ruled out as unlikely. If the phenomenon of an increase in the concentration of the lipid under discussion is not an experimental artifact, the presence of phospholipase D (phospholipase 4), catalyzing hydrolytic removal of alcohol from phosphoglyceride molecules, in the mitochondria must be postulated. The confirmation of this suggestion requires special investigations.

The LPO level in the mitochondria can therefore be regarded not only as a factor regulating the activity of their endogenous phospholipase A_2 [1, 6, 12], but also as an indicating factor, picking out among the mitochondrial phospholipids those objects for preferential phospholipase attack.

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EFFECT OF SYNTHETIC PHENOLIC ANTIOXIDANTS ON THE JUXTAMURAL pH OF THE GASTROINTESTINAL TRACT OF INTACT AND VAGOTOMIZED RATS

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The aim of this investigation was to study the effect of synthetic inhibitors of free-radical oxidation reactions belonging to the group of sterically screened phenols, fat-soluble (dibunol — 4-methyl-2,6-di-tert-butylphenol) and water-soluble [2,6-di-tert-butyl-4-(1-aminoethyl)phenol hydrochloride — BH-3], on the juxtamural pH (JpH) profile of the digestive tract in rats under normal and pathological conditions. Such an investigation is necessary because in recent years antioxidants (AO) have begun to be used for the treatment of diseases of the digestive system [1, 5-7], whereas their effect on the structure and functions of the digestive organs under normal and pathological conditions has not been studied. As the criterion for evaluation of the action of AO on function of the gastrointestinal tract, we used a physiological parameter, namely the hydrogen ion concentration in the paramucosal (juxtamural) layer (JpH). The pathological state of the digestive system was simulated by subdiaphragmatic division of the vagus nerves. When this model was chosen the following factors were taken into consideration: 1) the craniobulbar portion of the CNS, represented by the vagus nerves, is the main source of parasympathetic innervation of the digestive organs [9]; 2) vagotomy is widely used in the surgical treatment of duodenal ulcer [12].

EXPERIMENTAL METHOD

Experiments were carried out on 150 male albino rats weighing 150-210 g. In series I the experiments were performed on initially intact animals (n = 103), of which 21 rats received a single injection of high doses of the compounds (45 mg/kg dibunol in a 3% solution of Tween-80 and 40 mg/kg of BH-3 in physiological saline, intraperitoneally), and the investigation was carried out 24 h later; 68 rats received the compounds in therapeutic doses (20 mg/kg dibunol and 25 mg/kg BH-3) and were investigated 7, 15, and 30 days after the first injection (the doses of the compounds were recommended by the Sector of Kinetics of Chemical and Biological Processes, Institute of Chemical Physics, Academy of Sciences of the USSR); in the control, 14 rats received 1 ml of a 3% solution of Tween-80 intraperitoneally, daily for 8 days. In series II rats subjected to bilateral subdiaphragmatic truncal vagotomy served as the test object. Starting from the 2nd day after the operation they were given the compounds in therapeutic doses and were investigated 7, 15, and 30 days after vagotomy. Under urethane anesthesia (150 mg/100g) JpH of the experimental and control animals of both series was measured 24 h after the last injection of the compound and after feeding, in the fundus of the stomach, the duodenum, jejunum, ileum, cecum, and rectum, by the method in [8]. Values of the gastroduodenal and ileocecal gradients (the coefficients, E_1 and E_2) were

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