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PHOSPHOLIPID HYDROLYSIS AND Fe^{++}-INDUCED CHEMILUMINESCENCE OF RAT LIVER MITOCHONDRIA DURING SURVIVAL *IN SITU*

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KEY WORDS: survival of organs; hydrolysis of phospholipids; chemiluminescence.

Anoxia of any origin is accompanied by disturbance of the structure and function of cell organelles as a result of hydrolysis of the membranes by endogenous phospholipases [8, 13, 15]. This applies chiefly to mitochondria, the degree of integrity of which will be the main factor predetermining the reparative potential of the cell. The effectiveness of restoration of structural and functional integrity of the cell after reoxygenation will depend not only on the degree of preceding hydrolysis of membrane lipids, but also on many other factors, including the level of lipid peroxidation (LPO). Investigations have shown accumulation of primary and secondary products of LPO in lipids extracted from the mitochondrial and microsomal fractions of liver in ischemia, even without reoxygenation; at the same time the level of antioxidative activity (AOA) was observed to fall [1, 2, 5, 10, 11]. However, in these experiments the oxygen balance of the tissues was not monitored and it is not clear whether conditions of anoxia were reached. Moreover, activation of LPO may have taken place during extraction of the lipids. Nevertheless, these results may be evidence if not of activation of LPO in the tissues during ischemia, at least of an increase in the activity of pro-oxidant factors in cell membrane lipids. At the same time there is evidence that AOA may increase in the cell during anoxia. For instance, lengthening of the latent period and a decrease in the intensity of Fe^{++}-induced chemiluminescence (Fe^{++}-CL) have been observed in homogenates or mitochondrial fraction during long periods (3-24 h) of survival of the rat liver in situ [6], and ascorbate-dependent LPO in liver homogenates has been found to be reduced in ischemia [11]. Estimation of the LPO capacity of the mitochondria at the time of onset of irreversibility of anoxic liver damage is particularly interesting. For

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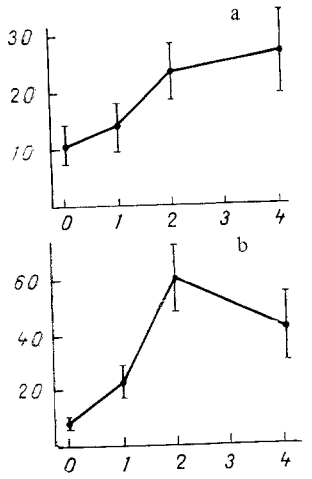

Fig. 1

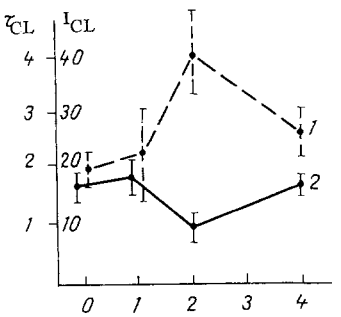

Fig. 2

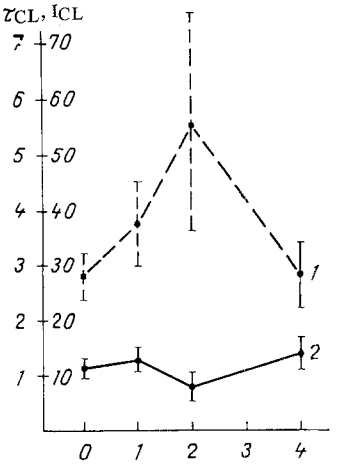

Fig. 3

Fig. 1. FFA content in mitochondrial (a) and light mitochondrial (b) fractions of rat liver cells depending on duration of survival of organ. Abscissa, time (in h); ordinate, FFA concentration (in nmoles/mg protein).

Fig. 2. Changes in period of development (1) and value of maximum of chemiluminescence (2) of mitochondrial fraction of liver depending on duration of survival of organ. Here and in Fig. 3: abscissa, survival time (in h); ordinate, period of development of CL (τ_{CL}, in min) and peak value of CL (I_{CL}, in relative units).

Fig. 3. Changes in period of development (1) and peak value (2) of chemiluminescence of light mitochondrial fraction of liver depending on duration of survival of organ.

the surviving rat liver the critical point of reversibility is about 2 h [14].

The aim of the present investigation was to study relations between the degree of phospholipid hydrolysis and the state of LPO by the Fe^{++}-CL method in two mitochondrial fractions of rat liver after survival of the organ for 1, 2, and 4 h. To assess the state of mitochondrial function the rate of oxygen consumption by the organelles was studied in different functional states as described by Chance.

EXPERIMENTAL METHOD

Noninbred male albino rats or August rats weighing 120-140 g were deprived of food for 12-24 h before the experiments. The animals (2-4 rats) were decapitated and the liver removed after the cadaver had been kept at 22-25°C for 1, 2, and 4 h. Mitochondria were isolated by differential centrifugation in medium of the following composition: sucrose 0.125 M, KCl 0.055 M, KH_2PO_4 0.02 M, EDTA 10^{-4} M, pH (0-1°C). Nuclei and cell fragments sedimented at 600 g for 10 min. The mitochondrial fraction was obtained by centrifugation of the supernatant at 8000g for 10 min. After sedimentation of the mitochondria the supernatant was again centrifuged at 18,000g for 10 min, yielding the light mitochondrial fraction in the residue. The isolated fractions were kept at 0°C. To rule out any effect of the organelle isolation procedures on CL, mitochondrial fractions were isolated from the liver of animals of the control group simultaneously.

The concentration of free fatty acids (FFA) was measured by the method of Anderson and MacCarty [12]. The protein concentration was determined by the biuret method.

Polarographic changes in the oxygen concentration were produced by means of a closed Clark's electrode (Radiometer, Sweden). Mitochondria, in a concentration of 1.0-2.0 mg protein/ml, were incubated in isolation medium to which were added 2 mM $MgCl_2$ and 10 mM Na succinate or a mixture of Na glutamate with Na malate to 4 mM, and 150-300 μM ADP. In the experiment, the mitochondria were passed successively through Chance's states 4_q-3-4_r-5 [7].

TABLE 1. Parameters of Functional State of Albino Rat Liver Mitochondria after Different Periods of Survival of the Organ *in Situ* (M ± m)

Parameter	Respiration substrate	Time, h		
		1	2	4
Rate of respiration in Chance's state 3 ($\bar{V}_3$), nanoatoms O_2/mg protein/min	Succinate	173±25 (5)	32±2 (4)	18±2 (4)
	Glutamate + malate	141±13 (7)	31±2 (4)	11±1 (4)
Rate of respiration in state of quiescence	Succinate	48±9 (5)	14±2 (4)	15±2 (4)
($\bar{V}_4^q$), nanoatoms O_2/mg protein/min	Glutamate + malate	33±7 (7)	15±2 (4)	7±1 (4)
Rate of respiration at rest ($\bar{V}_4^n$), nanoatoms O_2/mg protein/min	Succinate	66±10 (4)	30±4 (4)	—
	Glutamate + malate	48±9 (7)	25±3 (4)	—
$AC = \frac{\bar{V}_3}{\bar{V}_4^q}$	Succinate	3,6	2,3	1,2
	Glutamate + malate	4,3	2,1	1,6
$RCC = \bar{V}_3/\bar{V}_4^0$	Succinate	2,6	1,1	—
	Glutamate + malate	2,9	1,3	—

Legend. Number of experiments given in parentheses.

CL was measured by an instrument of the usual type consisting of glass cuvette with a working volume of 10 ml and a glass mixer, FEU-39 photomultiplier, amplifier, power source, and automatic writer.

The incubation medium in the course of the experiment was: KCl 0.115 M, inorganic phosphate 0.02 M, pH 7.4. To induce CL, $FeSO_4$ was added in a concentration of 10^{-3} M.

EXPERIMENTAL RESULTS

The experiments showed that the FFA concentration in mitochondrial and light mitochondrial fractions increased from 11 to 28 and from 8 to 43 nmoles/mg protein/4 h of survival of the liver respectively (Fig. 1), evidence of hydrolysis of about 10% of the phospholipids. The fact that FFA accumulation was more marked (approximately twice) in the light mitochondrial fraction may be the result either of a higher content of phospholipase A_2 in the light mitochondrial fraction, or a decrease in the sedimentation constant of the injured mitochondria, because of which they are isolated in the light mitochondrial fraction.

In the course of survival of the organ a considerable decrease in the rate of oxidation of the mitochondria took place in various functional states (Table 1), and the respiratory control coefficient (RCC) became minimal after only 2 h. The acceleration coefficient (AC) proved to be a more stable parameter and fell significantly only toward 4 h of survival. Such a difference between RCC and AC is difficult to explain, but this phenomenon is characteristic of injured mitochondria [7].

During the study of Fe^{++}-CL of the mitochondrial fractions the following parameters were examined: latent period, period of development of the "slow flash," and peak intensity of the "slow flash."

The latent period of Fe^{++}-CL in each concrete experiment was always greater than in the parallel control, although their absolute values varied considerably from one experiment to another. Mean values of latent period, however, in both mitochondrial fractions of rat liver cells were increased by 2-4 times during survival (Table 2).

Results reflecting changes in parameters of the slow flash of chemiluminescence of the mitochondrial fractions are given in Figs. 2 and 3. They show that the longest duration and lowest peak value of Fe^{++}-CL occurred after survival of the liver for 2 h; after survival for 1 and 4 h these parameters differed only a little from the control values.

Interpretation of the results of the study of Fe^{++}-CL is often inconsistent, due to several factors. Its parameters depend in general on the presence of both groups of factors, namely pre-oxidants and antioxidants, in the test system [3, 4]. Under the experimental

TABLE 2. Dependence of Latent Period of Fe^{++}-CL of Mitochondrial Fractions on Duration of Survival (M ± m)

Fraction	τ_e/τ_c		
	1 h	2 h	3 h
Mitochondrial	1,84±0,11 (9)	2,51±0,18 (3)	2,64±0,59 (5)
Light mitochondrial	1,89±0,07 (8)	4,13±0,10 (3)	2,81±0,16 (3)

Legend. τ_e and τ_c denote latent periods of Fe^{++}-CL in experiment and control respectively. Number of experiments shown in parentheses.

conditions used, the difference observed in the parameters of chemiluminescence between the control and experimental samples evidently indicates a change in the ratio between the pro- and antioxidant properties of the organelles in the course of survival of the liver in favor of antioxidants, at least for a 2-h period of survival, when all three parameters of Fe^{++}-CL differed from the control values.

The nature of the antioxidants appearing in the mitochondria during survival of the liver in situ is not clear. It can be tentatively suggested that the antioxidants either are not set free, or they lose their activity in the course of lipid extraction, for as results obtained by other workers have shown, AOA in hydrophobic extracts of their mitochondria is reduced at all periods of survival in experiments conducted under similar conditions. Anti-oxidants are perhaps hydrolysis products of nonlipid components of mitochondria or salts of FFA [9]; moreover, the possibility cannot be ruled out that anti-oxidants may be released from "physiological depots" or activity of relatively inactive antioxidants may be increased.

The increase in AOA of the mitochondria is probably a unique response of the cell to anoxia, aimed at preventing general poisoning of the animal by LPO products on reoxygenation.

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FLUOROMETRIC SCOPOLETIN PEROXIDASE METHOD OF MONOAMINE OXIDASE ASSAY IN HUMAN PLATELETS

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KEY WORDS: monoamine oxidase; platelets, schizophrenia.

Monoamine oxidase (MAO) plays a key role in the metabolism of biogenic amines, for it catalyzes oxidative deamination of these compounds [2]. Interest in the study of human platelet MAO is due to the disturbance of some characteristics of this enzyme in various diseases [8, 10-12]. Besides nerve endings and other monoamine-containing cells, platelets take part in processes connected with monoamine metabolism, and for that reason they can be regarded as a convenient model with which to study the transport, storage, metabolism, and receptor functions of these compounds [10]. Disturbance of monoamine metabolism plays a leading role in the pathogenesis of some mental diseases [10]. Accordingly the study of the properties of human platelet MAO is a promising trend in psychiatry and, in particular, in the study of the pathogenesis of schizophrenia, alcoholism, and certain types of depression and drug addiction [8, 10-12]. Platelet MAO is also a convenient model for studying the mechanisms of action *in vivo* of the MAO inhibitors used in psychiatry [10]. All these circumstances point to the need for a study of the properties of human platelet MAO under normal and pathological conditions, but unfortunately this is difficult because of the low specific activity of this enzyme in human platelets. The highly sensitive radiometric methods usually adopted for this purpose are not always available and have certain disadvantages inherent in methods of amine oxidase assay based on measurement of the concentration of aldehydes formed, for these compounds are very labile and reactive [2]. Existing fluorometric methods of MAO assay in human platelets as a rule enable only one substrate to be used, and this substantially reduces the value and informativeness of the results, especially in pathological states [2].

This paper describes a method of determining MAO activity in human platelets developed by the writers on the basis of the scopoletin peroxidase method of determination of low concentrations of H_2O_2 described in the literature [6]. Certain enzyme characteristics were studied in normal subjects and schizophrenics, and our own data were compared with results obtained by other methods.

EXPERIMENTAL METHOD

The control group consisted of healthy men and women aged from 18 to 26 years (nine persons). The other group consisted of patients with acute manifestations of hallucinatory-paranoid schizophrenia (six men and one woman) aged from 24 to 41 years.

Blood was taken from the cubital vein into a plastic vessel containing 0.6 ml of 0.27 M EDTA (pH 7.4) to 20 ml blood. Platelets were obtained by the method described previously [3] with the following modifications. Erythrocytes and leukocytes were sedimented at 300g for 5 min (4°C). Platelet-enriched plasma was centrifuged at 1100g for 20 min at 4°C to

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