# ROLE OF ENDOGENOUS FREE IRON IN ACTIVATION OF LIPID PEROXIDATION DURING ISCHEMIA

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Lipid peroxidation (LPO), plays an important role in cell damage associated with ischemia [2, 5-7, 9, 12]. In ischemia activity of superoxide dismutase and of other antioxidant enzymes declines [7], whereas phospholipase activity rises [11, 12], and this may lead to activation of LPO [12]. There is also evidence in the literature that endogenous iron may participate in the activation of LPO in ischemia [8]. Some of the iron contained in the cell has been shown to be in a dissolved state, in the $Fe^{++}$ form [14], i.e., in the form in which iron activates LPO in model systems [5, 15]. The aim of this investigation was to study the role of free iron in LPO activation in ischemia.

## EXPERIMENTAL METHOD

Male Wistar rats weighing 150-200 g were used. After decapitation laparotomy was performed on the rats, a catheter was introduced into the superior vena cava, and the liver was perfused with physiological saline to remove blood. As a model of ischemia the liver was removed and placed in a bottle containing 3 ml physiological saline. The bottle was closed and incubated at 20 or 37°C.

Concentrations of total intracellular and free cytoplasmic iron in the liver were determined by the EPR method [13]. To determine cytoplasmic free iron a piece of living weighing 1 g was placed in a test tube and covered with 0.25 $cm^3$ of 50% sodium nitrite. The contents of the tube were allowed to stand for 20 min at room temperature. The liver was then placed in special molds [4] and frozen in liquid nitrogen. The samples thus obtained, identical in shape and size, were transferred to a Dewar vessel with finger-shaped side-tube which was placed in the resonator of an EPR spectrometer. EPR spectra were recorded on a Varian E-40 spectrometer under the following conditions: frequency of klystron generator 100 kHz, amplitude of modulation 3 G, time constant 1 sec, scanning speed of spectrum 100 g/min, power of SHF field 5 mW, temperature 77°K.

To determine the free iron content in micrograms per gram wet weight of liver a known quantity of $Fe^{++}$ in the form of a solution of $FeSO_4 \cdot 7H_2O$ was added to the liver homogenate and its concentration measured by the EPR method, after which a graph of amplitude of EPR signal versus quantity of added iron was plotted.

The quantity of malonic dialdehyde (MDA) and lipid hydroperoxide was determined by the method in [10]. Lipids were extracted from the liver by Folch's method. The concentration of diene conjugates was determined from optical density at 232 nm, as in [5], and standardized relative to weight of lipids.

## EXPERIMENTAL RESULTS

In the model of ischemia at 20°C the MDA concentration increased as early as after 2 h of incubation, and after 6 h its concentration was twice the initial level (Fig. 1A). Incubation at 37°C led to an increase in the rate of MDA accumulation in the liver; significant differences from the initial level were observed as early as 1 h after the liver had been put into a state of ischemia. After the 6th hour of incubation the MDA concentration was almost 4 times higher than initially (Fig. 1A). Parallel with the MDA level in the liver the total

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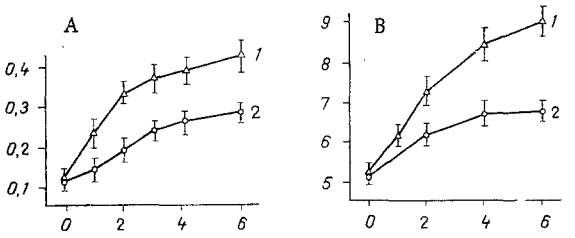


Fig. 1. Kinetics of accumulation of MDA (A) and total free iron (B) in rat liver during ischemia. Abscissa, duration of ischemia (in h); ordinate; A) MDA level (in μM), B) iron content (in μg/g liver).

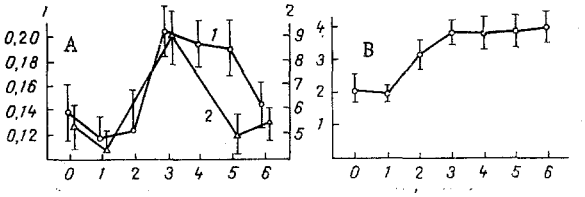


Fig. 2. Changes in concentrations of hydroperoxides, diene conjugates (E) and cytoplasmic free iron (B) in rat liver during ischemia. Abscissa, time (in h); ordinate: A) level of hydroperoxide (in μM) and diene conjugates (in $D_{232}$/mg lipids); B) iron level (in μg/g liver). 1) Hydroperoxides, 2) diene conjugates (in $D_{232}$/g lipid). $D_{232}$ denotes optical density at 232 nm.

concentration of free iron also was measured. The kinetic curves of free iron accumulation in the tissue at 20 and 37°C were found to be similar to the corresponding curves of MDA accumulation (Fig. 1B). This points to a connection between the two processes.

Later, to clarify the picture, a more detailed study was made of ischemia at 20°C. Concentrations of primary LPO products namely diene conjugates and lipid hydroperoxides, were measured after different times of ischemia (Fig. 2A). During the first few hours of incubation the concentration of primary LPO products changed in accordance with a kinetics similar to that of the change in levels of MDA and total free iron (compare Fig. 2 with Fig. 1B, 2): during the first hour no change was observed, but later the levels of both diene conjugates and lipid hydroperoxides rose rapidly until the 3rd-4th hours of incubation. After this, however, unlike MDA and free iron, the concentration of primary LPO products fell, to reach the initial level by the 6th hour. This development can be explained as follows. On addition of iron to a lipid-containing system in concentrations exceeding 58 μM, it decomposes lipid hydroperoxides to MDA [5]. Later the concentrations of MDA and hydroperoxide remain unchanged because iron in these concentrations exhibits the properties of an antioxidant [5]. A similar picture also was observed in the present experiments. It can be tentatively suggested that the concentration of accumulated free iron after 3-4 h of incubation exceeded 58 μM, lipid hydroperoxides were decomposed to MDA, and LPO processes were arrested.

At the same time, the picture observed can be explained by oxidation of the readily oxidized fraction of lipids in the liver by the 3rd-4th hour. In this case the quantity of primary LPO products would fall immediately, and this will be followed by a fall in the contents of secondary products later [5].

It was shown in [13] that the free iron pool consists of two parts: One part is contained in closed membranous structures, the rest is dissolved in the cytoplasm. The results show that with an increase in the duration of ischemia the quantity of free iron in the cytoplasm rises (Fig. 2B). Comparison with the curves of accumulation of total free iron (Fig. 1A, 2) and of cytoplasmic iron showed that the course of the changes in parallel. The difference between the levels of total and cytoplasmic iron, reflecting the quantity of free iron contained in membranous structures, was unchanged after different times of incubation. It can accordingly be postulated that this subpool of free iron does not participate in LPO activation.

Every $Fe^{++}$ ion decomposes one molecular of hydroperoxide, and in turn it is converted into $Fe^{+++}$. One of the oxidation products of hydroperoxides is MDA which, when formed, is

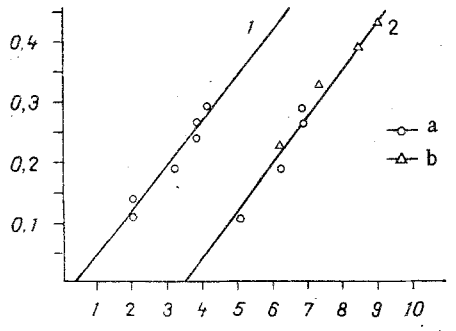


Fig. 3. Dependence of MDA concentration in rat liver during ischemia on level of free iron accumlating in it. Abscissa, iron level (in μg/g liver); ordinate, MDA concentration (in μM). 1) Cytoplasmic free iron, 2) total free iron. a) At 20°C, b) at 37°C.

metabolized by the body. Hence it follows that, other conditions being equal, the stationary free iron concentration in the tissue must be proportional to the stationary MDA concentration. Of course this relationship must exist if free iron participates in LPO activation. Dependence of the quantity of MDA accumulating in the tissue after different times of ischemia on the total free iron level and on the cytoplasmic free iron level is illustrated in Fig. 3. In the first case, the straight line calculated for the points by the method of least squares, intersects the X axis at a point corresponding to 3.5 g iron/g wet weight of liver (Fig. 3). This iron concentration corresponds to the subpool bound with membrane structures [13]. The other straight line, drawn through points obtained for cytoplasmic iron, intersects the X axis close to zero, evidence that the whole subpool of cytoplasmic iron participates in LPO activation during ischemia. It is probably this part of the free iron which regulates LPO processes in the cell, including under normal conditions.

The concept that the free iron level in the cytoplasm depends on the reducing potential of the cell was formulated by A. F. Vanin. This hypothesis has been confirmed by a number of investigations [3, 15]. On the basis of the results of these investigations and our own data the mechanism of LPO activation in ischemia can be represented as follows: 1) In the absence oxidation of substrates in the cell ceases; 2) reduced substrates accumulate in the cell and the reducing potential of the cell rises; 3) the increase in reducing potential of the cell leads to liberation of iron from ferritin; 4) the released iron enters the cytoplasmic subpool of free iron; 5) activation of LPO by $Fe^{++}$ ions takes place.

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# MICROCHROMATOGRAPHIC STUDY OF HIPPOCAMPAL AREA CA3 PROTEINS DURING PROLONGED POST-TETANIC POTENTIATION IN SURVIVING SLICES

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The study of the molecular mechanisms of plasticity, the connection between electrical activity and metablism in neurons, and the dependence of protein synthesis in postsynaptic cells on the character of stimulation is one of the principal tasks in neurochemistry. The most adequate model for the analysis of these mechanisms is the phenomenon of prolonged post-tetanic potentiation (PPTP), developing in the hippocampus as a result of short high-frequency stimulation [2, 6, 7].

Most neurochemical studies of PPTP have been undertaken on whole hippocampal slices, consisting of a highly heterogeneous cell population. It is only by the use of micromethods that it has been possible to study α-motoneurons of the cat spinal cord under conditions of post-tetanic potentiation of monosynaptic reflexes, and to discover, by microdisk electrophoresis, changes in the spectrum of water-soluble proteins located in the zone of fast-migrating fractions, including brain-specific protein S-100 [1].

The study of synthesis of proteins and, in particular, of brain-specific proteins in a homogeneous population of postsynaptic cells during the development of PPTP is of undoubted interest. By using a system of synaptic connections (granule cells of the dentate gyrus — pyramidal cells of area CA3) incorporation of $^3$H-leucine into water-soluble protein of this zone has been investigated during the development of PPTP (in surviving slices after stimulation of mossy fibers) [1].

## EXPERMENTAL METHODS

Experiments were carried out by the method in [13] with certain modifications. Adult Wistar rats were decapitated under chloroform anesthesia, the hippocampus was removed, transverse slices 300-400 μ thick were cut, and placed in a thermostatically controlled chamber with a continuous flow of liquid (Yamamoto's medium), aerated with 95% $O_2$ and 5% $CO_2$.

Electrolytically sharpened bipolar tungsten electrodes with a distance of 50 to 300 between tips were used for stimulation. Tetanization was undertaken with a group of square pulses (50-300 Hz, duration 0.05-1 msec, 20-25 V) for 10-15 sec. Global activity was recorded by a glass electrode (diameter of tip 3-10 μ), filled with Yamamoto's medium.

After stimulation the slices were incubated for 1 h at 37°C in Yamamoto's salt solution containing 100 μCi/ml of [$^3$H]leucine (specific radioactivity 60 Ci/mole, from Amersham Corporation (England), and control "unpotentiated" slices were placed in a similar solution. At the end of incubation the slices were washed 3 times with ice-cold salt solution to remove extracellular label. Area CA3 was removed under a binocular loupe in cold extraction buffer (a 1% solution of Tween in Tris-phosphate buffer, pH 7.4), homogenized in the cold for 2 min with a mechanical tissue microhomogenizer at 2000 rpm, incubated for 12 h at 4°C, and centrifuged for 1 h at 1800g. The protein concentration in the supernatant was estimated from specific binding of protein with Coomassie blue G-250 [12].

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