ROLE OF FREE Fe^{++} IN LIPID PEROXIDATION DURING ISCHEMIA AND REOXYGENATION OF THE LIVER

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Tissue damage during ischemia and reoxygenation is linked with two processes, which disturb the integrity of cell membranes. First, in oxygen deficiency the intracellular endogenous phospholipases are activated [2, 5, 12]. Second, in anoxic states lipid peroxidation (LPO) is intensified [8-10]. The cause of phospholipase activation has been shown to be a disturbance of intracellular Ca^{++} transport [5, 13]. Intensification of LPO is associated with decreased activity of various antioxidative enzymes of the cell [1, 8], and also with accumulation of free Fe^{++} in the tissue during eschemia [6, 7, 13]. However, the study of the role of Fe^{++} in LPO activation during ischemia either has yielded only very indirect data, or the periods of ischemia investigated were rather long in duration [7]. The role of Fe^{++} in reoxygenation-induced tissue damage has not been discussed.

The aim of the present investigation was accordingly to study the role of free Fe++ in intracellular LPO activation in the liver in a model of ischemia and reoxygenation.

EXPERIMENTAL METHOD

Slices 0.5 mm thick and measuring 2 × 2 mm were cut from the perfused rat liver. The model of ischemia was created by incubating thin slices of rat liver in physiological saline in an atmosphere of nitrogen. Sections incubated in an atmosphere of air served as the control. As the model of reoxygenation sections were incubated for 30 min under nitrogen, and then for 30 min in air. Another model of ischemia also was used: a rat was anesthetized with hexobarbital and the liver perfused for 1 min with physiological saline; a ligature was then tied around the upper left lobe of the liver for 30 min. The lobe was excised 30 min later and the free Fe^{++} concentration in it was determined.

To determine the free Fe^{++} concentration samples of thin slices (volume 1.5 ml) or of whole liver (weight 1 g) were treated with a 50% solution of sodium nitrate (0.5 ml) and a 10% solution of ascorbic acid (0.2 ml) by the method in [11]. Sections treated with sodium nitrate were incubated for 20 min at 20°C. The EPR spectra of the samples were recorded at the temperature of liquid nitrogen on a Varian E-4 spectrometer. The amplitude of the low-field component of the EPR spectrum with g = 2.03 was considered, as described in [11], to be proportional to the concentration of free Fe^{++}, dissolved in the cell cytoplasm. Besides the Fe^{++} dissolved in the cytoplasm (Fe_c), cells also contain [3, 11] free Fe^{++} in membrane compartments (Fe_m). The total free Fe^{++} (Fe_t^{++}), i.e., the sum of Fe_c^{++} and Fe_m^{++}, was determined by the same sequence of procedures as for Fe_c^{++}, except that after incubation for 20 min at 20°C the samples were incubated for a further 7 min at 45°C. Incubation at 45°C [3, 11] leads to release of Fe^{++} from the membrane compartments into the cytoplasm. The absolute content of Fe^{++} in the samples were determined from a calibration curve, plotted after the addition of different amounts of Fe^{++} to a tissue homogenate, followed by measuring the EPR signal of the nitrosyl complexes.

To determine the malonic dealdehyde (MDA) concentration in the liver slices and incubation medium the contents of the flasks were centrifuged for 15 sec at 600g and 0.5 ml of the supernatant was transferred into a test tube with 2 ml of 5 mM Tris-buffer (pH 7.5), 0.5 ml TCA, and 0.1 ml of ionol (10^{-2} M). The slices were washed twice with 5 volumes of Tris-buffer (5 mM, pH 7.5), and sedimented each time by centrifugation. After the second washing

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TABLE 1. Free Fe^{++} and MDA Concentrations during Oxygenation, Ischemia, and Reoxygenation, Modeled on Thin Liver Slices (M ± m)

Experimental conditions	Fe_t^{++}, μg/g weight	MDA_{total}	MDA_{tissue}	MDA_{medium}
		μM		
Initial level	5,07±0,23	0,97±0,30	0,030±0,006	0,015±0,003
Oxygenation for 30 min	6,09±0,50	2,91±0,45	0,039±0,015	0,074±0,009
Ischemia for 30 min	6,23±0,59	2,91±0,39	0,096±0,023*	0,030±0,005*
Oxygenation for 60 min	5,59±0,34	3,21±0,33	0,043±0,017	0,084±0,007
Ischemia for 60 min	6,73±0,68*	4,12±0,42*	0,153±0,036*	0,024±0,009*
Reoxygenation for 30 + 30 min	4,77±0,55	5,27±0,55*	0,057±0,021	0,108±0,003*

Legend. Here and in Table 2: *$p < 0.01$ compared with corresponding control.

the slices were treated with 0.1 ml of 10^{-2} M ionol and the volume of the sample was made up to 3 ml with Tris buffer. The slices were then homogenized and centrifuged for 10 min at 1800g, after which 2 ml of supernatant was transferred into a test tube with 1 ml of 20% TCA.

All the samples were then centrifuged for 10 min at 1800g; 2 ml of the supernatant was then mixed with 1 ml of 0.5% 2-thiobarbituric acid (TBA) and the mixture incubated for 15 min at 100°C [4]. The absorption spectrum of the samples in the 500-600 nm region was then recorded on a Beckmann DU-7 spectrophotometer. By the use of two basic wavelengths (515 and 570 nm) the amplitude of the peak with a maximum at 532 nm on the spectrum was measured.

The MDA concentration was calculated by assuming that the coefficient of molar extinction of complexes of MDA with TBA is $1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ [4].

EXPERIMENTAL RESULTS

Experiments on thin slices showed that 30 min of ischemia or oxygenation leads to an increase in the MDA concentration in both cases (Table 1). No difference was observed between these tests. Consequently, this fact is not the result of absence of oxygen in the medium. After 1 h of ischemia more MDA accumulated in the samples than during oxygenation, and more accumulated during reoxygenation than during ischemia. This result is in agreement with the data in [1, 10], showing that LPO is activated by ischemia and reoxygenation.

Changes in the MDA concentration in the washed thin liver slices and in the incubation medium showed that during ischemia most LPO products accumulated in the tissue. As regards oxygenation and, in particular, reoxygenation, in these cases most MDA accumulates in the incubation medium. This is probably the result of more severe damage to the cell membranes when oxygen is present in the medium. This fact may be evidence of the leading role of LPO in damage to cell membranes.

Measurements of the Fe^{++} concentration showed that it was present only in the tissue, and no nitrosyl complexes could be detected in the incubation medium.

The free Fe^{++} concentration increased during the first 30 min of incubation during both ischemia and oxygenation, but as in the case of MDA, no difference was observed between the samples. After 1 h of incubation the free Fe^{++} concentration became higher during ischemia than during oxygenation. Thus a parallel increase in the MDA and free Fe^{++} concentrations was observed, as also was demonstrated previously during long (over 1 h) periods of ischemia [7].

By contrast with this, during reoxygenation the MDA concentration rose, but that of free Fe^{++} fell. It can be tentatively suggested that free Fe^{++} is oxidized during reoxygenation, activating LPO. To determine the exact causes of LPO activation during reoxygenation, we studied the Fe_c^{++} concentration after 30 min of ischemia and oxygenation, i.e., before the beginning of reoxygenation. The results showed that while the Fe_t^{++} level was the same, the Fe_c^{++} level was higher during ischemia than in the oxygenated samples (Table 2). In other words, under ischemic conditions part of the free Fe^{++} passes from membranous structures into the cytoplasm: decompartmentalization of free Fe^{++} takes place. A similar result was obtained with whole liver after 30 min of ischemia.

On the basis of these data it is possible to explain LPO activation during postischemic reoxygenation. During reoxygenation oxygen is supplied to cells in whose cytoplasm the free Fe^{++} concentration is increased, and this causes marked intensification of LPO. The free Fe^{++} is oxidized, thereby activating LPO, and its concentration in the cell is reduced.

TABLE 2. Cytoplasmic and Total Free Fe^{++} Concentrations during Oxygenation and Ischemia for 30 min Modeled on Thin Liver Slices (M ± m)

Sample	Experimental conditions	Fe_c^{2+}	Fe_t^{2+}
Thin liver slices	Initial level	2,11±0,15	5,2±0,3
	Oxygenation for 30 min	2,40±0,30	6,7±0,7
	Ischemia for 30 min	3,40±0,33*	7,0±0,9
Whole liver	Control (intact liver)	1,55±0,20	5,8±0,7
	Ischemia for 30 min	2,84±0,33*	6,3±0,7

The question arises: do processes capable of disturbing the integrity of the membranous compartments of the cell take place in the tissues during ischemia? It was noted above that damage to cell membranes during ischemia is connected not only with the intensification of LPO, but also with activation of the intracellular phospholipases. It can be postulated that phospholipase activation is also a cause of destruction of the intracellular compartments of release of Fe^{++} into the cytoplasm.

The fact that these processes are coordinated in time is particularly interesting: both phospholipase activation [5, 2] and decompartmentalization of iron take place during the first 30 min of keeping the tissue in an anoxic state, whereas a longer period of time is required to release Fe^{++} from ferritin [3].

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