Effect of Transitory Ischemia on Liver Lysosomal Apparatus in Rats with Different Resistance to Hypoxia

O. R. Grek, A. B. Pupyshev, and E. V. Tikhonova

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We studied the state of lysosomal apparatus and pro- and antioxidant activity in the liver of rats with different resistance to hypoxia during postischemic recovery. Under normal conditions the lysosomal apparatus did not differ in highly and low resistant animals. During ischemia and reperfusion the damage to hepatic lysosomal membranes in rats highly resistant to hypoxia was less pronounced than in low resistant animals. These differences also concerned labilization of lysosomes during exposure to damaging factors (hypotonia and Triton X-100). The rats highly resistant to hypoxia differed from low resistant animals by higher stability of lysosomal membranes, lower prooxidant activity (malonic dialdehyde content), and higher tissue concentration of α-tocopherol during reperfusion.

Key Words: *individual resistance to hypoxia; hepatic ischemia; reperfusion; liver lysosomal apparatus; oxidative stress*

Genetic and phenotypic mechanisms providing the resistance of organs and tissues to hypoxia attract much recent attention. The rats highly resistant (HR) and low resistant (LR) to hypoxia differ in the intensity of lipid peroxidation (LPO) and activity of the antioxidant system [1,4,7]. Little is known about the lysosomal apparatus of the liver in rats with different resistance to hypoxia.

Here we studied changes in the lysosomal apparatus of the liver, LPO intensity, and content of the natural antioxidant tocopherol in rats with different resistance to hypoxia under normal conditions and after ischemia-reperfusion of the liver.

MATERIALS AND METHODS

Experiments were performed on 96 male Wistar rats weighing 150-250 g. The resistance to hypoxia was determined as described elsewhere [1]. The experiment started not less than 1 month after group formation. Total liver ischemia was produced by occlusion of the hepatic artery and portal vein for 30 min with a microforceps (after separation of the bile duct). The animals were narcotized with ethaminal (40 mg/kg intraperitoneally) and operated under aseptic and antiseptic conditions. The measurements were performed on days 3, 7, and 14 of the postischemic period. The rats were decapitated under light ether anesthesia. Liver homogenates were prepared as described previously [9].

Activity of free lysosomal enzymes in liver homogenates was estimated immediately after their isolation. Activities of acid phosphatase, β-galactosidase, and acid RNase were measured routinely [6,8]. Free enzyme activity was expressed in percents of total activity. The state of lysosomal membranes in the liver was determined by free enzyme activity and its increase after treatment with a hypotonic sucrose or low concentrations of Triton X-100 [6]. Protein concentration in samples was measured by the method of Lowry [13].

Vitamin E content in the plasma was estimated by high-performance liquid chromatography with UV detection. Tocopherol concentration was measured spectrofluorometrically [10]. The content of malonic dial-

Department of Pharmacology, Central Research Laboratory, Novosibirsk State Medical Academy. ***Address for correspondence:*** nikagre@mail.cis.ru. Grek O. R.

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dehyde (MDA) in liver homogenates was determined in the reaction with thiobarbituric acid (TBA) [5].

The results were statistically processed. The arithmetic means (*M*) and errors (*m*) were calculated. The significance of differences was estimated using Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

Analysis of the functional state of the liver lysosomal apparatus at various terms after ischemia revealed an increase in free enzyme activity and a decrease in the resistance of lysosomal membranes to damage. Stability of lysosomal membranes differed in HR and LR rats (Tables 1 and 2).

During acute postischemic period, free acid hydrolase activity in LR animals increased from 18-22 to 35-40% of total activity; after hypotonic treatment (or exposure to low concentrations of Triton X-100) the increase in free enzyme activity reached 22-24% of total activity. Changes in the liver lysosomal apparatus in HR rats during the acute stage after ischemia-reperfusion were less pronounced. Free lysosomal enzyme activity in HR rats was lower than in LR animals (25-30% of the total activity). Lysosomal membranes were less sensitive to hypotonic sucrose solution; free enzyme activity increased only by 16-18%. Free enzyme activity in LR and HR rats remained high on day 7 of the postischemic period (33-41 and 22-31%, respectively). At this term the sensitivity of lysosomes to damaging factors tended to increase: free enzyme activity increased to a greater extent (especially in LR rats, 30-36% of the total activity on day 7 *vs*. 19-24% in HR animals). In HR rats the involvement of lysosomes in the recovery of the liver after ischemia-reperfusion was accomplished by the 14th day of the postischemic period. At this term free enzyme activity approached the control level. In LR animals signs of activation of the lysosomal apparatus were observed on day 14 after reperfusion of the liver.

These results suggest that HR and LR rats differ by the reaction of liver lysosomes to ischemic injury. HR rats are characterized by higher stability of lysosomal membranes during the early postischemic period compared to LR animals.

Published data show that different resistance of lysosomal membranes to reperfusion injury in HR and LR rats is associated with different activities of tissue antioxidant systems [2,4,7]. On days 3 and 14 of the reperfusion period the increase in the content of TBA-reactive substances in liver homogenates from LR rats 1.9 and 2.1 times surpassed the corresponding para-

TABLE 1. Free β-Galactosidase, Acid Phosphatase, and Acid RNase Activities in Liver Homogenates from Rats with Different Resistance to Hypoxia during Reperfusion (% of Total Activity, *M*±*m*)

Parameter		Control	Reperfusion period, days		
			3	7	14
β-Galactosidase	LR	21.6±1.6	40.3±2.4*	40.6±1.5*	35.3±3.1*
	HR	19.8±1.4	31.2±0.6*+	31.40±1.25*+	20.2±1.5+
Acid phosphatase	LR	22.4±1.7	35.3±1.9*	35.5±1.1*	32.3±0.7*
	HR	21.1±2.2	31.0±0.5*	25.8±2.5*+	28.0±1.8*+
Acid RNase	LR	18.3±1.5	35.9±1.8*	33.3±2.4*	27.3±1.1*
	HR	18.2±0.8	25.5±0.8*+	22.7±1.6*+	19.9±1.0+

Note. Here and in Table 2: $p<0.05$: *compared to the control; +compared to LR rats.

TABLE 2. Increase in Free β-Galactosidase, Acid Phosphatase, and Acid RNase Activities after Treatment of Liver Homogenates with Hypotonic Sucrose Solution (% of Total Activity, *M*±*m*)

Parameter		Control	Reperfusion period, days		
			3	7	14
β-Galactosidase	LR	15.8±0.8	23.4±0.6*	29.9±3.0*	28.6±2.9*
	HR	13.4±0.7	18.7±0.9*+	20.8±1.6*+	19.0±1.0*+
Acid phosphatase	LR	14.6±1.4	24.1±2.2*	32.7±1.7*	27.5±0.8*
	HR	13.7±1.3	18.1±0.4*	19.9±1.5*+	21.8±0.6*+
Acid RNase	LR	14.7±0.2	22.6±0.7*	35.5±1.4*	24.3±2.6*
	HR	14.0±0.6	16.6±0.5*+	22.1±2.0*+	19.5±0.9+

TABLE 3. Changes in the Contents of MDA and α-Tocopherol in the Liver and Plasma of Rats during Reperfusion (n=6-8, $M\pm m$)

Parameter	Control		Reperfusion in the postischemic period, days			
			3		14	
	LR	HR	LR	HR	LR	HR
MDA in homogenate, μmol/mg protein	30.7±1.7	25.0±2.0^{x}	56.3±2.3*	38.4±2.6*^{x}	40.7±2.8*	29.7±2.2^{x}
α-Tocopherol in homogenate, μg/mg protein	5.7±0.6	6.7±0.5	2.4±0.4*	3.9±0.5^{x}	4.4±0.5^{+}	7.1±0.6^{x}
α-Tocopherol in plasma, nmol/ml	1.78±0.23	1.49±0.44	1.03±0.15	1.34±0.29	1.11±0.16	0.73±0.26

Note. p<0.05: *compared to the control; $^{+}$compared to day 3 of reperfusion; ^{x}compared to LR rats.

meters in HR animals. During reperfusion of ischemic liver the content of α-tocopherol in HR rats at these terms was higher than in LR animals (Table 3). These peculiarities provided effective control over oxidative destruction of membrane structures during the reperfusion period. In LR rats α-tocopherol concentration in the liver during reperfusion was 1.6 times lower than in HR animals (p<0.05). This determines high prooxidant activity and low resistance of lysosomal membranes of rats LR to hypoxia.

In conclusion, the state of liver lysosomes in rats with different resistance to hypoxia did not differ under normal conditions. However, changes in the lysosomal apparatus of liver cells during reperfusion differed in HR and LR rats. HR animals probably possess a more adequate adaptive mechanism, which reduces ischemic damage to lysosomal membranes of liver cells. This decreases the severity of injury to cells and subcellular structures and accelerates recovery of the liver.

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