STRUCTURAL AND FUNCTIONAL ASPECTS OF POSTISCHEMIC REGENERATION OF THE RAT HEPATOCYTE ENDOPLASMIC RETICULUM

V. I. Sharapov, I. A. Sokirchenko, O. R. Grek, V. A. Shkurupii, and A. V. Dolgov

UDC 616.36-005.4-036.8-07:616.36-018.1:576.311.336

KEY WORDS: ischemia of the liver; xenobiotic metabolism; ultrastructure.

Several investigations have shown a disturbance of the structure and decrease in the activity of enzyme systems of the hepatocyte endoplasmic reticulum after acute interruption of the blood flow into the liver [4, 11]. However, the consequences of acute ischemia of the liver during the postischemic period have received less study.

The aim of this investigation was to study activity of microsomal mono-oxygenase complexes and ultrastructure of rat hepatocytes in the early and late stages after total ischemia of the liver for 30 min.

EXPERIMENTAL METHODS

Experiments were carried out on 200 male Wistar rats weighing 150-240 g. Total ischemia of the liver for 30 min and a mock operation were carried out by the method described previously [2].

Microsomes were isolated from the liver by differential centrifugation [1]. The concentration of cytochromes P-450 and b_5 [7], and the velocity of the aminopyrine-N-demthylase [9] and aniline-p-hydroxylase reactions [5] were determined in the microsomal fraction the 1st, 3rd, 7th, 14th, and 21st days of the postischemic period. Cytochrome concentrations were recorded on a Hitachi-356 differential spectrophotometer (Japan). The ability of phenobarbital (PB) to induce activity of microsomal hydroxylases was assessed after its administration for 4 days (50 mg/kg body weight once daily), before decapitation of the animals on the 7th and 14th days of the postischemic period. The protein concentration in the microsomes was determined by Lowry's method [6]. Optical density was measured on a Specol-21 spectrophotometer (East Germany).

Samples of liver were obtained 24 h and 3, 7, and 14 days after ischemia of the liver for 30 min, fixed in 1% OsO_4 solution in phosphate buffer, and embedded in Epon. Ultrathin sections were stained with a saturated aqueous solution of uranyl acetate, and with lead citrate, and examined in the JEM-100 electron microscope. Twenty areas of cytoplasm were photographed in hepatocytes from each animal and investigated morphometrically in accordance with the recommendations in [12]. Differences between mean values were compared with a $P < 0.05$ level of significance.

EXPERIMENTAL RESULTS

The concentration of cytochrome P-450 was unchanged 24 h after restoration of the blood flow, but the concentration of cytochrome b_5 at this time was 12.5% lower than in the control (Table 1). The cytochrome concentrations on the 3rd day of the postischemic period were 34.4 and 29.6% lower respectively than the control. On the 7th and 14th days the cytochrome P-450 concentration in the microsomes was 37.1 and 31.2% lower respectively, and the control level was reached on the 21st day. The cytochrome b_5 concentrations were restored on the 14th day of the postischemic period.

Department of Pharmacology, Central Research Laboratory, Novosibirsk Medical Institute.
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, NO. 3, pp. 277-279, March, 1986. Original article submitted January 25, 1985.

TABLE 1. Time Course of Concentrations of Cytochromes P-450 and b_5 and Changes in Velocity of Microsomal Hydroxylation of Aminopyrine and Aniline in Postischemic Period (M ± m)

Parameter	Experimental conditions	Recovery period, days					Control (intact animals)
		1	3	7	14	21	
Cytochrome P-450	Mock operation	0,936±0,05	0,959±0,06	0,812±0,08	0,989±0,08	0,964±0,09	0,989±0,06
	Ischemia	0,864±0,05 (87,4)	0,649±0,02* (65,6)	0,622±0,04* (62,9)	0,680±0,05* (68,8)	1,005±0,10 (102)	
Cytochrome b_5	Mock operation	0,628±0,02	0,628±0,04	0,555±0,03*	0,683±0,04	0,670±0,05	0,679±0,02
	Ischemia	0,594±0,02* (87,5)	0,478±0,02* (70,4)	0,437±0,02* (64,3)	0,647±0,01 (95,3)	0,653±0,05 (96,2)	
Aminopyrine N-demethylation	Ischemia	1,613±0,06* (80,6)	0,934±0,09* (46,7)	1,234±0,07* (61,7)	1,415±0,03* (70,8)	2,041±0,26 (102)	2,001±0,13
Aniline p-hydroxylation	Ischemia	0,283±0,01* (58,0)	0,168±0,03* (34,4)	0,165±0,02* (33,8)	0,196±0,01* (40,2)	0,364±0,02* (74,6)	0,488±0,03

Legend. Concentrations of cytochromes given in nmoles/mg protein. Percentages of control in parentheses. Number of animals 8-14. Here and in Table 3: velocity of aminopyrine N-demethylation given in nmoles HCHO/min/mg protein, velocity of aniline-p-hydroxylation in nmoles p-aminophenol/min/mg microsomal protein; $P < 0.05$ compared with control.

TABLE 2. Results of Morphometry of Cytoplasmic Structures of Rat Hepatocytes (M ± m)

Parameters studied	Control	Time after removal of clamp, days			
		1	3	7	14
Smooth endoplasmic reticulum					
V_V	2,51±0,32	1,81±0,21	3,27±0,38	2,08±0,30	2,67±0,27
S_v	1,17±0,13	0,85±0,08*	1,18±0,11	1,03±0,11	0,62±0,07*
Rough endoplasmic reticulum					
V_V	19,20±0,95	16,50±0,67*	16,45±0,63*	11,87±0,72*	15,46±0,84*
S_V	4,82±0,24	5,20±0,20	4,31±0,18	4,73±0,28	4,77±0,19
s_V	0,251±0,018	0,315±0,018*	0,262±0,015	0,398±0,034*	0,309±0,021*
Attached ribosomes (N_A)	30,7±2,56	18,7±1,20*	14,6±1,0*	19,8±1,70*	14,2±1,10*

Legend. V_V) Bulk density on ultrastructues (in % of volume of cytoplasm); S_V) surface density of membranes of ultrastructures (in μ^2/μ^3 of cytoplasm); N_A) density (number) of structures per square micron area of section through hepatocyte cytoplasm; s/v) surface-volume ratio (ratio of surface area of structures to its volume). Liver from five animals studied in control and at each period after ischemia. Asterisk indicates significant difference from control.

TABLE 3. Changes in Rate of Microsomal Metabolism of Aminopurine and Aniline in Postischemic Period during Induction of Mono-Oxygenase by PB (M ± m)

Parameter	Intact animals	Induction by PB (control)	Postischemic period	
			ischemia of liver plus induction by PB	
			7 days	14 days
Aminopyrine N-demethylation	2,260±0,22 (6)	5,085±0,49 (8)	2,795±0,20* (7)	3,762±0,18* (8)
Aniline p-hydroxylation	0,546±0,05 (6)	1,015±0,08 (8)	0,472±0,03* (7)	0,712±0,03* (8)

Legend. Number of animals shown in parentheses.

The velocity of the aminopyrine-N-demethylase reaction fell from the 1st until the 3rd day of the postischemic period (by 53.3% of the control). Activity of aminopyrine-hydroxylating enzymes gradually returned to its initial level on the 21st day of the postischemic period (Table 1). The limitation of the velocity of the aminopyrine-N-demethylase reaction observed in the recovery period corresponding to a fall in the cytochrome P-450 concentration in the microsomes at all periods studied after ischemia of the liver. Consequently, it can be postulated that the factor limiting the velocity of N-demethylation of aminopyrine in the postischemic period is the cytochrome P-450 concentration. The velocity of the aniline-p-hydroxylase reaction in the postischemic period was inhibited by a greater degree than the fall in the cytochrome concentration, namely by 30-40% of the initial level throughout the recovery period, and it remained 25.4% below the control value on the 21st day after ischemia (Table 1). It can be concluded from these data that the factor limiting the velocity of the ainiline-p-hydroxylase reaction in the postischemic period is not the concentration of cytochrome P-450, but probably a decrease in its catalytic activity against type II substrates (aniline). The cause of the changes mentioned above may be disturbances of the structure of the microsomal membrane, into which the hemoprotein is incorporated [3].

Histological study of liver sections showed no necrotic changes in the hepatocytes at any period of the investigation. According to the electron-microscopic data, the surface area of membranes of the smooth endoplasmic reticulum was less than in the control 1 and 14 days after ischemia of the organ. Profound changes took place in the structural organization of the rough endoplasmic reticulum (RER). Besides changes in the total volume, fragmentation of the tubules of RER was observed, as was also shown by an increase in the ratio of the surface area of the tubules of RER to their volume on the 1st, 7th, and 14th days after ischemia of the liver (Table 2). More definite evidence of changes in structures of the microsomal membranes was given by a persistent decrease in the number of adherent ribosomes in the postischemic period.

Injection of PB, the mechanism of whose inducing action includes activation of synthesis of enzyme proteins and microscomal membranes [8, 10], was accompanied by only a very small increase in the rate of metabolism of aminopyrine and aniline on the 7th and 14th days of the postischemic period (Table 3). This can be taken as evidence of the inability of the protein-synthesizing system to respond adequately to the inducing action of PB, evidently due to a marked decrease in the number of ribosomes in the polysomes of RER during these and the preceding periods after ischemia of the liver.

Thus during 2-3 weeks of the recovery period changes in the concentration of microsomal cytochromes and in activity of hydroxylase reactions are linked with structural changes in the endoplasmic reticulum in this period.

LITERATURE CITED

1. A. I. Archakov, Microsomal Oxidation [in Russian], Moscow (1975).
2. O. R. Grek, V. I. Sharapov, V. S. Litvinov, and A. V. Dolgov, Farmakol. Toksikol., No. 3, 114 (1984).
3. V. V. Lyakhovich and I. B. Tsyrlov, Structural Aspects of Biochemistry of the Mono-Oxygenases [in Russian], Novosibirsk (1978).
4. M. E. Ferrero, R. Orsi, and A. Bernelli-Zazzera, Exp. Mol. Pathol., 28, 256 (1978).
5. Y. Imai, A. Ito, and R. Sato, J. Biochem. (Tokyo), 60, 417 (1966).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
7. T. Omura and R. Sato, J. Biol. Chem., 239, 2370 (1964).
8. S. Orrenius, J. L. E. Ericson, and L. Ernster, J. Cell. Biol., 25, 627 (1965).
9. E. A. Smucler, E. Arrenius, and T. Hultin, Biochem. J., 103, 55 (1967).
10. W. Stäubli, R. Hess, and E. R. Weibel, J. Cell. Biol., 42, 92 (1969).
11. B. R. Trump, I. K. Berezesky, and A. R. Osornio-Vargas, in: Cell Death in Biology and Pathology, London (1981).
12. E. R. Weibel, Stereological Methods, Vol. 1, Practical Methods for Biological Morphometry, London (1979).