EFFECT OF HYPOTHERMIA ON METABOLISM IN THE LIVER DURING PRESERVATION

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The effect of hypothermia to different depths (18-20°C and 4-6°C) on carbohydrate metabolism and the degree of solubilization of the enzymes in the liver (lactate and glutamate dehydrogenases, urocaninase, deoxyribonuclease, glucose-6-phosphatase) during preservation of the unperfused organ was studied in 20 experiments on dogs. The effectiveness of preservation was assessed during subsequent normothermic perfusion for 2 h. After preservation of the liver at 18-20°C marked solubilization of the above-mentioned enzymes was observed, indicating a disturbance of the integrity of the cell membranes during preservation. After preservation of the liver at 4-6°C moderate utilization of the glycogen reserves in the liver and of sugar in the perfusion fluid was observed, suggesting uniform depression of metabolism in the liver and predominance of normal tissue respiration over glycolysis on restoration of the circulation in the organ. KEY WORDS: hypothermia; rat liver; glycolysis; respiration; blood flow.

Lengthening the period of maintenance of the function of isolated organs, or their preservation, is an important problem in transplantology. Hypothermia is most frequently used as a method of preservation of organs [2, 9, 13]. However, deep hypothermia leads to a deficiency of energy which is necessary for balancing diffusion processes in the tissues. The intensity of these processes is reduced by a lesser degree than the intensity of metabolism [7], thus leading to edema and death of the cells.

The best hypothermic regimes for the kidneys are now known [7], but the dependence of the degree of anoxic damage to the liver and of depression of metabolism in that organ on different temperature regimes has received comparatively little study.

The object of this investigation was to study changes in carbohydrate metabolism and permeability of the cell membranes in the liver during preservation of the organ at different temperatures.

EXPERIMENTAL METHOD

Experiments were carried out on 20 mongrel dogs. After removal of the liver from the donor, its tissue was washed to remove blood cells and filled with perfusion fluid, the composition of which was worked out in the writers' laboratory. The liver was then kept for 3 h in a refrigerator, filled with the same perfusion fluid. This period of 3 h is in practice the time required to prepare a recipient for the operation of liver transplantation.

In the experiments of group 1 (107 dogs) the liver was preserved at 18-20°C, in those of group 2 (10 dogs) at 4-6°C. After 3 h the liver was connected to an artificial circulation apparatus and subjected to normothermic perfusion [3] for 2 h in order to verify the maintenance of its function after preservation. The period of stabilization of the blood flow and temperature in the liver in the two groups of experiments was 45-60 min and the temperature of the perfusion fluid was kept constant at 37°C. In the course of perfusion the volume velocity of the blood flow was 0.7 ml/g/min; the pressure in the portal vein 100-120 mm water, the pH 7.34-7.48, the hematocrit index 16-17%, and the O_2 difference in blood entering and leaving the liver 20/30% H_2O.

After laparotomy, before removal of the liver from the donor animal, and again during maintenance of an artificial circulation in the liver, after the end of each hour of perfusion, marginal biopsy of the liver was carried out and samples of perfusion fluid were tested. The glycogen content in the liver tissue was determined

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TABLE 1. Dynamics of Indices of Carbohydrate Metabolism in Perfusion on Fluid and Liver Tissue after Preservation at Different Temperatures (M ± m)

Group of expts.	Temp. of preservation	Stages of experiments	Indices of carbohydrate metabolism in perfusion fluid				Liver glycogen
			sugar	lactic acid	pyruvic acid	lactate/pyruvate ratio	
1	18—20°C	During stabilization of blood flow	154,6±29,3*	200±13,4	117±3,3*	174±37*	79±4,6
		At end of perfusion	150,2±28,6	212±12,9*	153±2,3*	147±26*	83,5±12,3
2	4—6°C	During stabilization of blood flow	90,8±5,18*	116±14,4*	173±3,05*	87,2±11,8*	79,3±11,4
		At end of perfusion	107,4±6,14	127,8±24,5*	192,5±10,4*	79,8±6,3	80±17,6

*Here and in Table 2 $P < 0.05$ compared with corresponding control.

[5]. The activity of the following enzymes was studied in liver homogenate prepared under standard conditions in 0.25 M sucrose: lactate dehydrogenase (LD) [2], glutamate dehydrogenase (GD) [12], urocaninase [1], deoxyribonuclease (DNase) [4], and glucose-6-phosphatase (G6P) [14]. The activity of these same enzymes and the concentrations of sugar [6], lactic acid [8], and pyruvic acid [10] were studied in samples of perfusion fluid. To determine unprecipitated enzyme activity some of the homogenate was centrifuged at 105,000 g. The unprecipitated activity was expressed as a percentage of the total activity of the corresponding enzymes. The initial level of unprecipitated activity for urocaninase, GD, and DNase was 17.7 ± 2.9, 4.3 ± 0.60, and 19.5 ± 1.7% respectively.

Because of the considerable difference between the initial statistics for carbohydrate metabolism and the variability of enzyme activity in the total homogenate and perfusion fluid, and also to exclude the factor of variation within groups, values were calculated as percentages of the initial data in each experiment. Subsequent statistical analysis of the results was carried out in relation to percentage deviations from the initial results.

EXPERIMENTAL RESULTS

As will be clear from Tables 1 and 2, in the experiments of groups 1 and 2 the glycogen level fell toward the period of stabilization. Glycogen metabolism in the two groups of experiments clearly followed different pathways. Glycogenolysis after preservation at 18-20°C was accompanied by an increase in the lactic acid and sugar concentrations in the perfusion fluid (Table 1). However, under these circumstances the liver G6P activity remained unchanged (Table 2). The results suggest the existence of two pathways for glycogen breakdown. The increase in the sugar concentration without activation of G6P indicates a hydrolytic pathway of catabolism, whereas the sharp rise in the lactic acid concentration and the lactate/pyruvate ratio point to the presence of a phosphorolytic pathway. Disproportionate depression of different stages of carbohydrate metabolism after preservation at 18-20°C was manifested as exhaustion of the glycogen reserves and failure to utilize the excess of sugar of the perfusion fluid for the energy requirements of the liver toward the time of stabilization of the blood flow in the organ. However, attention is drawn to maintenance of an unchanged glycogen content in the liver tissue and the constant sugar concentration in the perfusion fluid toward the end of normothermic perfusion, compared with their levels during the period of stabilization of the artificial blood flow. The increase in the pyruvic acid content against the background of low LD activity does not rule out the formation of energy reserves through activation of gluconeogenesis.

After preservation of the liver at 4-6°C, by contrast with the experiments of group 1, the sugar concentration in the perfusion fluid remained practically unchanged and substantial inactivation of G6P was observed (Tables 1 and 2). This may indicate that the liver preserved its ability to utilize the sugar of the perfusion fluid when the blood flow was restored. Toward the end of normothermic perfusion a sharp decrease was found in the lactate/pyruvate ratio although the initial level of LD activity was maintained in the tissue. It can tentatively be suggested that under these conditions normal tissue respiration in the liver predominated over glycolysis.

Investigation of enzyme activity in the perfusion fluid showed an increase in both groups of experiments toward the period of stabilization of the blood flow in the liver. However, as is clear from Table 1, preservation at 18-20°C led to greater escape of enzymes into the perfusion fluid. The sharp rise in the activity of the mitochondrial enzymes will be noted: GD, urocaninase, and lysosomal DNase. Toward the end of normothermic perfusion marked inactivation of GD and DNase with a simultaneous increase in their unprecipitated activity

TABLE 2. Enzyme Activity after Preservation of Liver at Different Temperatures (M ± m)

Grp. of expts.	Temp. of preservation	Material studied	Type of activity	Stages of experiments	Activity of enzyme				
					urocaninase	DNase	LD	GD	G6P
1	18—20°C	Perfusion fluid	Activity in perfusion fluid (in % of initial level)	During stabilization of blood flow	330±46,6*	196±13,8*	197±16,2*	311,8±32,8*	—
				At end of perfusion	370±28*	221±9,2	248±25,1	495,8±32,3*	—
			Total activity (in % of initial level)	During stabilization of blood flow	94±1,12*	88±12,6	90±8,49	124±15,7	104,8±6,3*
		Tissue		At end of perfusion	101±1,0	79,1±5,8*	83,8±4,5*	93,6±1,1*	122,1±11,4*
		Tissue	Unprecipitated activity (in % of total activity)	During stabilization of blood flow	21,5±0,98	32,8±2,08	—	15,37±2,6*	—
				At end of perfusion	20,8±2,3	49,2±6,1	—	20,5±4,17*	—
2	4—6°C	Perfusion fluid	Activity in perfusion fluid (in % of initial level)	During stabilization of blood flow	130±25,5*	148±8,3*	128,7±14,7*	222±12,9*	—
				At end of perfusion	191,5±36,3*	191,6±29,4	237±13,7	270±29,2*	—
		Tissue	Total activity (in % of initial level)	During stabilization of blood flow	106,7±1,8*	99,3±1,3	87,6±4,03	105,8±9,7	73±6,2*
				At end of perfusion	99,2±2,5	94,6±3,3*	100±0,0*	107,8±5,1*	67,2±6,2*
		Tissue	Unprecipitated activity (in % of total activity)	During stabilization of blood flow	20,8±3,5	41,4±5,7	—	6,84±0,8*	—
				At end of perfusion	29,3±3,5	44,5±7,1	—	9,97±3,3*	—

were observed at this period in the liver tissue. The permeability of the cell membranes in the liver tissue was perhaps increased considerably after preservation at 18-20°C leading to increased solubilization of the mitochondrial and lysosomal enzymes.

An increase in the permeability of the plasma membrane and a progressive reaction of the mitochondrial and lysosomal structures, manifested as substantial solubilization of enzymes, is a poor prognostic sign. Disproportionate depression of the activity of the various enzymes of carbohydrate metabolism after preservation of the liver at 18-20°C leads to imbalance of energy-forming reactions and aggravates the injury to the cell membranes.

After preservation of the liver at 4-6°C the increase inpermeability of the cell membrane and the labilization of the mitochondrial and lysosomal structures during the period of normothermic perfusion were less marked than in the experiments of group 1.

The results suggest that of the two temperature regimes compared, hypothermia to 4-6°C is better for short-term preservation of the liver.

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