Adaptation of the Membrane Receptor Apparatus of Blood Cells after the Combined Use of Cryodestruction and Plasma Beam in Experimental Cirrhosis of the Liver

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It is shown that cryodestruction combined with resection with a plasma beam aggravates alterations of the functions of the blood cell receptor complex affected by cirrhosis, leading to hypoinsulinemia, inhibited capacity of cells to utilize glucose, a lowered metabolic activity of insulin receptors, and other changes. Three weeks after surgery the energy metabolism of the blood cells returns to the normal level, while the activity of insulin receptors and of hexokinase exceeds the control level.

Key Words: *insulin receptors; glucose utilization; autoregulatory mechanism*

The liver possesses an autoregulatory mechanism that controls the rate of insulin breakdown depending on changes of the blood level of the hormone, including cases characterized by insufficient insulin production by β-cells [3]. In this connection it is worth noting that in many stress situations associated with insufficient hormone production the liver can play the main role in the regulation of both degradation and secretion of insulin, thus maintaining normoglycemia. Thus, in a stress situation the blood concentration of glucose, insulin, and other metabolites depends to a large degree on the function of the liver.

The goal of the present study was to see how dissection of the cirrhotic liver of experimental animals with a plasma beam after prefreezing the line of resection affects the status of the receptor apparatus of blood cells.

MATERIALS AND METHODS

The work was carried out on 40 chinchilla rabbits weighing 3-3.5 kg. A comparative assessment of the effect of cryodestruction, plasma beam resection, and a combination of these methods on the intact and cirrhotic liver was performed in 6 series of experiments. Experimental cirrhosis was induced by subcutaneous injection of 40% CCl_4 solution as described elsewhere [7]. All surgical procedures were carried out under hexenal anesthesia.

The status of erythrocyte receptors was estimated by the rate of glucose utilization from the incubation medium, hexokinase activity, and ATP content. The composition of the incubation medium corresponded to the conditions of the test [8]. The depletion of glucose from the incubation medium was estimated by the glucose-oxidase method using Boehringer kits. Hexokinase activity was measured by spectrophotometry in the glucose-6-phosphate dehydrogenase system, recording the increment of extinction at 340 nm. To the main component of the medium used in this system [1] the following inhibitors of glycolytic reactions were added: monoiodoacetate (0.03 mM) and NaF (10 mM). The reaction was triggered by the introduction of erythrocyte lysates. ATP content was estimated using Boehringer kits.

The intensity of lipid peroxidation in the erythrocyte membranes was judged by the following parameters: level of malonic dialdehyde

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Table 1. Changes of Certain Properties of Erythrocytes after Combined Use of Cryodestruction and Plasma Scalpel Dissection of Cirrhotic Liver ($M\pm m$)

Parameter	Control	Time postoperation, days					
		1	3	7	14	21
IBA of erythrocytes, %	22.5±2.1 (15)	24.3±1.3 (14)	31.8±1.1* (14)	24.1±1.3 (10)	26.3±1.08 (12)	24.1±0.9 (10)
Glucose utilization, nmol/10^6 cells/hour	1.340±0.133 (9)	0.970±0.98 (8)	0.801±0.130* (8)	0.823±0.123* (9)	0.992±0.21 (9)	1.089±0.21 (9)
Hexokinase activity, μmol NADPH/min/g hemoglobin	1.72±0.09 (15)	1.61±0.17 (12)	1.12±0.18** (13)	1.31±0.15* (13)	1.57±0.11 (13)	2.13±0.13* (14)
ATP content, μmol/g hemoglobin	2.53±0.13 (17)	2.12±0.13 (15)	2.21±0.01** (15)	1.83±0.01** (15)	2.35±0.08** (14)	2.51±0.05 (13)

Note. *$p<0.05$; **$p<0.01$; number of experiments in parentheses.

(MDA), measured after Osakawa and Matsushita [14], level of hydroperoxides [2], and antioxidative activity (AOA) [6].

RESULTS

Earlier we showed that the metabolic changes observed in the blood during the first week after various influences upon the intact liver can result from the adaptive-compensatory reactions of the organism to various damaging effects ensuing from a variety of factors associated with the surgical intervention [5]. Plasma beam resection-induced hypoglycemia, hypoproteinemia, and increase in MDA concentration, and the hyperglycemia and hypoproteinemia that are observed after cryodestruction at later periods, are leveled in the case of the combined action of these surgical methods. The results of the present study show that administration of the hepatotropic toxin is followed by a drastic rise in MDA concentration (from the initial level of 3.51±0.11 to 10.63±0.566 nmol/ml; $p<0.02$) and a drop in serum AOA from 61.8±3.1 to 36.5±2.5% ($p<0.05$). All varieties of manipulations with cirrhotic liver during the whole follow-up period are associated with high levels of the parameters under study, e.g., one week after the surgical interventions hyperglycemia is observed (the glucose concentration increases from 3.8±0.6 to 6.1±0.9 mmol/liter; $p<0.05$); 3 weeks postoperation the MDA concentration remains elevated in both the serum and erythrocyte membranes in all cases; serum AOA is inhibited more than twofold ($p<0.001$). Thus, the combined use of cryodestruction and plasma beam resection does not inhibit the processes of free-radical lipid oxidation that accompany experimental cirrhosis. It is known that this may affect the status of the cellular receptors.

In fact, study of the insulin receptors in the plasma membrane of lymphocytes has shown that one day after cryogenic action and cryosurgery in combination with plasma beam resection the specific insulin binding by lymphocytes is reduced to 15.7% (control level 29.8%; $p<0.05$). On the 3rd day a regular rise in the insulin-binding activity (IBA) of lymphocytes is seen, a tendency which is characteristic for an inflammatory process. Toward the end of the 7th day the IBA drops again. The pathogenesis of the further decline of IBA is probably connected with the continuing increase of lipid peroxidation. This may in turn intensify the metabolic derangements.

The data concerning erythrocyte receptors after combined cryodestruction and resection of cirrhotic liver with a plasma beam are presented in Table 1. The most marked alterations in the receptor apparatus were observed 3 days after the operation. All analyzed parameters of erythrocyte membranes in experimental rabbits differ reliably from the control. Incubation of the cells in the medium with the optimal content of glucose (10 mM) and inorganic phosphate (14 mM) revealed a reduction of the rate of glucose utilization by erythrocytes of operated animals (combined action of cryodestruction and plasma-mediated resection).

Under physiological conditions the erythrocyte uptake of glucose depends on multiple factors, one of the important roles belonging to hexokinase. After the combined intervention, the hexokinase activity decreased dramatically and represented 65% of the initial level. Glucose utilization by erythrocytes dropped by 40.2% ($p<0.05$), and the ATP level by 13%. The normal rate of glucose uptake by erythrocytes after the combined action on the cirrhotic liver is impaired due to the decreased hexokinase activity; glucose phosphorylation is considered as the first limiting factor of glucose consumption by these cells.

Under physiological conditions the energy requirements of erythrocytes are fulfilled by glucose utilization in glycolysis and the pentose phosphate pathway. Perhaps, after combined action the retardation in the substrate output for these conversions in the hexokinase reaction affects the total rate of ATP accumulation in the erythrocytes. Moreover, the drop in hexokinase activity leads to a reduced use of ATP for glucose-6-phosphate generation. It is worth noting that the degree of inhibition of insulin receptor kinase declines reduced with the rise in ATP concentration [13]. The lowering of hexokinase activity is apparently related to the changes in membrane structure, as is evidenced by an intensification of lipid peroxidation [6]. Reduced ATP production and activated lipid peroxidation in the cells lead to alterations in the structural-functional properties of membranes and insulin receptors. Decline of AOA may cause inhibition of ATP generation, destruction of the membrane lipid bilayer, disturbance of selective permeability, and changes of the functional status of receptors, including the insulin receptors.

The slow normalization of the analyzed parameters of membrane status proves the severity of the pathological shifts associated with the surgical intervention and with the serious damage to membranes mediated by the liver damage. The derangement of liver function leads to the development of hyperinsulinemia [11]. Plasma membranes of the liver in rats with CCl_4-induced experimental cirrhosis bind smaller quantities of insulin [13] and bear insulin receptors with a lowered affinity for insulin as compared to the membranes of the control animals. Erythrocytes of patients with chronic hepatitis and cirrhosis of the liver also exhibit reduced numbers of insulin receptors [12]. However, the changes observed in receptor activity are unlikely to be due to hyperinsulinemia, since no changes were found in the activity of insulin receptors on adipose cells in the patients with cirrhosis and hyperinsulinemia; moreover, the content of insulin receptors on monocytes and erythrocytes of patients with cirrhosis is reduced against the background of both hyper- and normoinsulinemia [10]. Induction of hepatitis by administration of D-galactosamine to intact rats and animals with streptozotocin-induced diabetes causes a lowering of the insulin receptor concentration on the liver plasma membranes in both groups of animals, while hyperinsulinemia develops only in the first group [9]. The development of high binding activity of insulin receptors after operation-associated stress is apparently caused on the one hand by hypoinsulinemia resulting from the stress, and on the other by functional insufficiency of the insulin receptors.

On the 7th day after the combination of cryodestruction and plasma surgery the levels of glucose utilization and of hexokinase activity remained virtually unchanged, whereas the ATP level showed a further decline, although IBA was within the normal range at all times of observation (or even elevated - on the 3rd day postoperation, Table 1). Thus, the results obtained provide evidence of metabolic abnormality of the insulin receptors. Fourteen days after the plasma surgery the levels of glucose utilization and of hexokinase activity in rats with cirrhosis were restored to the normal level, but the ATP content remained reliably lower than in the control. Three weeks after the operation both glucose utilization and ATP content normalized. The activity of insulin receptors of the blood cells and of hexokinase even exceeded the control levels.

Thus, the results of our investigations show that cryodestruction in combination with plasma-mediated resection leads to a further increase of cirrhosis-induced alterations of the functions of the blood cell receptor apparatus, including hypoinsulinemia, a reduced ability of cells to utilize glucose, a lowering of the metabolic activity of insulin receptors, a rise in the intensity of lipid peroxidation in the blood cell membranes, and other disorders. Three weeks after the operation the energy metabolism of cells returns to the normal level. Moreover, at this time the activity of insulin receptors and of hexokinase exceeds the control values.

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Effect of Weightlessness on the Development of the Nervous System and Peripheral Analyzers in *Xenopus laevis* Larvae

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The study was carried out within the framework of a Russian-Canadian experiment aboard the Bion-10 satellite. The volume and surface area of the gray and white matter and ventricles of the brain, retina, olfactory placodes, the VIII nerve ganglia, and vascular plexus were measured in *Xenopus laevis* which had been in a state of weightlessness for 2 weeks since their hatching. Zero gravity was found to stimulate the growth of nerve processes, to increase the surface of the vascular plexus, and to impede the development of the retina, olfactory placodes, and VIII nerve ganglia.

Key Words: *weightlessness; nervous system; peripheral analyzers; Xenopus laevis*

Human and animal organisms adapt to microgravitational conditions during long space flights. Adaptation involves primarily changes in the circulation and in mineral and tissue metabolism [1,3,6], but the adaptive changes of fully formed organisms differ radically from the adaptation of embryos, fetuses, and larvae of vertebrates. There are several reasons for this. Development is associated with active cell proliferation and the formation of rudiments of organs and systems. The instability of these processes is well known and is responsible for many congenital diseases and developmental abnormalities. On the other hand, mineral and tissue metabolism is more labile in a growing organism than in an adult. This imparts specific features to the mechanisms of adaptation to zero gravity.

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The informative value of experiments investigating the development of vertebrates under conditions of weightlessness is obvious, but poses quite a number of problems as well. Mammalian embryos are hardly fit for this purpose because of the intrauterine development of warmblooded animals. The effects of a maternal organism are hard to differentiate from the direct effects of weightlessness on the embryo. That is why autonomously developing amphibian embryos appear to be promising research objects, for the direct influence of weightlessness on their development can be detected.

The present research was carried out within the framework of the joint Russian-Canadian experiment *Development* devoted to a comprehensive study of the morphogenesis of *Xenopus laevis* larvae. In this study we investigated the effect of an 11-day stay under conditions of weightlessness on the development of the nervous system, peripheral analyzers, and vascular plexus of the brain. Hatch-

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