Effects of Long-Term Alcoholic Intoxication, Ethanol Withdrawal, and Insulin on Glucose Utilization, Hexokinase Activity, and ATP Level in Rat Erythrocytes

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Erythrocytes from rats that have been receiving ethanol for 30 days show a reduced capacity to utilize glucose and a reduced hexokinase activity. Following ethanol withdrawal, glucose utilization by erythrocytes and their hexokinase activity do not change significantly, while the ATP level in these cells drops. Insulin administered to the rats after ethanol withdrawal normalizes glucose utilization and ATP and raises hexokinase activity to levels exceeding its control values.

Key Words: *erythrocytes; alcohol; insulin*

One of the most specific indicators of chronic alcoholic intoxication is abnormal erythrocyte structure. The alterations undergone by erythrocytes in alcoholism result from the direct impact of ethanol and its metabolites on cells and reflect the state of the organs where they are produced and decomposed. It is therefore hard to overestimate the importance of studies on the properties of erythrocytes for diagnosing alcoholism, for gaining knowledge about the time course of pathological processes in this state, and for elucidating the efficacy of various therapeutic agents. However, because the available laboratory/clinical and experimental information on the metabolism of red blood cells and on the state of the hemopoietic organs in alcoholism is fragmentary and contradictory, it is not possible to evaluate unambiguously the direction and course of the pathological changes brought about by ethanol in these cells. Also, there are very few studies addressing the effects of ethanol withdrawal on erythrocyte metabolism. Furthermore, virtually nothing is known about the effects exerted on erythrocytes by pharmacological agents that can produce specific therapeutic effects in alcoholics.

The purpose of this study was to examine some parameters of energy and carbohydrate metabolism in erythrocytes of rats in the course of long-term alcohol intoxication and during the withdrawal period and to see how insulin administered to rats after the prolonged alcohol intoxication might influence these parameters.

MATERIALS AND METHODS

The study was carried out on random-bred male rats during a single season. Alcoholic intoxication was induced by repeated intragastric administration of a 40% ethanol solution at a rate of 4 g/kg body weight. Erythrocytes for analysis were taken from the blood in the following six groups of rats after their decapitation: untreated controls (group 1); rats given ethanol for 15 days (group 2); those given ethanol for 30 days (group 3); those that had received ethanol for 30 days and were decapitated on

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the 4th or 9th day after its withdrawal (groups 4 and 5, respectively); and those that had received ethanol for 30 days followed by daily insulin injections at 3 IU/kg for 3 days (group 6). Rats of groups 2, 3, and 6 were decapitated 24 h after the last ethanol or insulin administration.

The state of the erythrocytes was assessed by their utilization of glucose and their hexokinase activity and ATP concentration.

The incubation medium used to estimate glucose utilization has been described previously [9]; the decline in glucose concentration was determined by means of the enzyme kit GOD-POD-Glucose Test (Finland).

Hexokinase activity was measured spectrophotometrically in a glucose-6-phosphate dehydrogenase system from the increment of extinction at 340 nm. The medium used for these measurements [1] was supplemented by the glycolysis inhibitors monoiodoacetate (0.03 mmol/liter) and sodium fluoride (10 mmol/liter). The reaction was started by adding erythrocyte hemolysate to the medium.

ATP was determined by a chemiluminescence method [2] in a modification that involved the use of external ATP standards. For its determination, the ATP reagent luciferin-luciferase (manufactured by Immolyum, Russia) was diluted to a concentration of 1 mg/ml in 0.1 mol/liter Tris-acetate buffer, pH 7.6, containing 10 mmol/liter $MgSO_4$. In the recording cuvette, 1 ml of this extract was mixed with 0.02 ml of a perchlorate extract of erythrocytes in a 1:1 ratio; in the 3 control cuvettes, the respective ATP standards were mixed with the same components. The ATP concentration was estimated by comparing the signal from the test sample with the nearest signal from one of the ATP standards.

RESULTS

The metabolic characteristics of erythrocytes are shown in Table 1. The greatest alterations were noted in erythrocytes of rats given ethanol for 30 days (group 3): in this group, all three parameters of erythrocyte metabolism differed significantly from their control values. The 15-day alcoholization did not induce substantial changes in these parameters. The reported information regarding the state of erythrocytes during the early phase of chronic alcoholization is scarce and contradictory, but the metabolic response of erythrocytes has been consistently shown to depend on the initial resistance of their membranes. For the recognition of latent alcoholism it is important to know when the primary liquefying action of ethanol on the erythrocyte membranes will cause a compensatory increase in their viscosity [15]. The results of this study indicate that the metabolic reorganization of erythrocytes, which was unidirectional due to similar conditions of their membranes in all test rats, was complete after 30 days of intoxication.

The finding that the erythrocytes from rats treated with alcohol for 30 days (group 3) utilized glucose at a significantly decreased rate for incubation in a medium with optimal glucose and inorganic phosphate concentrations (12 and 15 mmol/liter, respectively) agrees with the reports that chronic alcohol consumption may result in diminished glucose utilization in the brain [18], skeletal muscles [13], and liver [16].

Under physiological conditions, glucose consumption by erythrocytes depends on many factors, an important one being the hexokinase of these cells. In our study, hexokinase activity fell markedly to 58% of its control value after 30 days of ethanol intake. The reduced glucose consumption was bound to affect the ATP level, which decreased by 16% over that period.

A similar effect of ethanol on the ATP level has been observed in human patients suffering from alcoholism, but not in the rat model of alcoholism [10]. Since disorders of energy metabolism in erythrocytes are always accompanied by alterations in their shape and size, our results are also consistent with numerous reports of the predominance of atypical erythrocytes in the blood of alcoholics. Possibly, the morphological changes in erythrocytes caused by alcohol are associated with biochemical processes in these cells that deplete them of energy.

Erythrocyte-related manifestations of ethanol exposure include stimulation or (in severe cases) inhibition of erythropoiesis, megaloblastic transformation, development of acanthocytosis, stomatocytosis, and other adverse effects [11].

Although the bone marrow retains in alcoholism its capacity to produce normal erythrocytes [7], all blood cells suffer direct toxic effects of ethanol, whose primary targets are the cell membranes. Alterations in the lipid composition of erythrocyte membranes and in the activity of membrane-associated enzymes have been cited as causes of abnormal properties displayed by these cells [3].

It follows, then, that the observed disturbances of carbohydrate and energy metabolism in erythrocytes during long-term alcoholic intoxication should be evaluated taking into consideration the diversity and complex interrelationships of the factors contributing to these disturbances.

TABLE 1. Parameters of Carbohydrate and Energy Metabolism in Erythrocytes of Alcohol-Treated Rats. The Values are Means±SEM

Parameter	Control	15 days of treatment	30 days of treatment	Day 4 after withdrawal	Day 9 after withdrawal	30 days of treatment + insulin for 3 days
Glucose utilization, nmol/10^6 cells/h	1.220±0.156 (n=8)	0.940±0.138 (n=7)	0.762±0.121* (n=9)	0.787±0.110* (n=10)	0.888±0.134 (n=8)	0.886±0.095 (n=9)
Hexokinase activity, μmol $NADPH_2$/min/g Hb	1.68±0.11 (n=12)	1.53±0.14 (n=7)	0.97±0.15** (n=11)	1.25±0.13* (n=10)	1.55±0.14 (n=8)	2.06±0.16* (n=8)
ATP level, μmol/g Hb	2.64±0.12 (n=14)	2.33±0.17 (n=8)	2.22±0.008** (n=14)	1.79±0.09** (n=11)	2.07±0.08** (n=8)	2.37±0.06 (n=8)

Note. Asterisks denote significant differences from control: *$p<0.05$, **$p<0.001$.

A factor preventing normal glucose uptake by erythrocytes in alcohol-treated animals is the reduced hexokinase activity, and the first limiting stage in the utilization of glucose is considered to be its phosphorylation [4].

Under physiological conditions, the energy requirements of erythrocytes are met through glucose utilization in the course of glycolysis and in the pentose phosphate pathway. During alcohol intoxication, slowed buildup of the substrate for these transformations in the hexokinase reaction possibly affects the overall rate of ATP accumulation in erythrocytes. An important effect on the ATP level appears to be exerted by the activation of Na,K-ATPase demonstrated for erythrocytes of alcoholized rats [8]. The membrane-damaging effect of ethanol, involving an increase in membrane permeability for sodium, is assumed to cause an adaptive boost of the work of the sodium-potassium pump with a consequent increase in the expenditure of ATP, which in turn contributes to the hexokinase deficiency. It is also possible that the reduction in hexokinase activity is directly associated with structural alterations in the cell membranes.

The slow normalization of the tested parameters of erythrocyte metabolism after the discontinuation of ethanol treatment provides convincing evidence for the profound pathological changes caused by alcoholic intoxication. By day 4 after ethanol withdrawal, despite a partial erythrocyte renewal in the circulation, glucose utilization and hexokinase activity had not increased significantly, while the ATP level had dropped. These results, which point to a metabolic slump of the bulk of cells during that period, may be interpreted in two ways. First, since alcohol has been shown to stimulate erythropoiesis up to a certain stage of the disease, the quantitative decrease in erythrocytes resulting from the shortened life-spans of these cells may be compensated for by their augmented production [5] and, if we assume that the relative proportion of old cells that have been subject to the direct toxic action of ethanol increases after ethanol withdrawal, then the observed changes in ATP levels may be thought to result from an altered state of the bone marrow (its altered productivity). The second explanation relies on the assumption that the metabolism of circulating erythrocytes is altered after ethanol withdrawal. During this period, the relationship between the energy-synthesizing and energy-expending processes is probably disturbed so that the latter processes predominate and the cells experience a deficiency in ethanol which they utilized as an important energy substrate during alcoholization. This assumption rests on the fact that a tricarboxylic acid cycle and vigorous synthetic reactions take place in the reticulocytes, which are produced in excess during alcoholic intoxication [5]. The most plausible explanation for the metabolic features of erythrocytes observed later after ethanol withdrawal is one that integrates the two explanations offered above. Thus, by day 9 after the discontinuation of alcohol intake, glucose utilization and hexokinase activity had both returned to normal, while the ATP levels remained significantly lower tna the control value. This period corresponds to that of complete erythrocyte renewal in the bloodstream of rats in health [7]. Our results indicate that abnormal cells were continuing to enter the bloodstream at that time.

Important findings appear to have emerged from our tests with insulin. After three daily insulin injections into rats that had been exposed to ethanol for 30 days, glucose utilization and ATP

returned to normal, while hexokinase activity even exceeded the control level.

There are indications in the literature that insulin is occasionally used in the treatment of alcoholism [2]. The mode of its therapeutic action has not been examined, but there is evidence that metabolic processes in the brain proteins of rats have been normalized by insulin administered to these animals after prolonged ethanol treatment [6]. The mechanisms of insulin action on erythrocytes requires special study. In addition to producing direct effects on these cells, insulin may alter erythropoiesis (directly or indirectly) in such a way that quantitatively altered erythrocytes will enter the blood. In any case, the results of this study suggest that insulin is effective in maintaining the viability of erythrocytes in alcoholic intoxication by activating their carbohydrate and energy metabolism.

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