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Cold Stress and Endogenic System Ethanol – Acetaldehyde

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Abstract

The article presents data on the effects of cold stress on changes in the concentrations of endogenous ethanol (EE) and acetaldehyde (EA), and the activities of the key enzymes involved in their metabolism—alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AHD) in laboratory animals. Mature male Wistar rats were used in the experiment. Animals in the vivarium were kept in cages at a temperature of 20°-22°C, without limitation of mobility, with free access to water with an adjustable light schedule (12 hours - light, 12 hours - darkness). Simulation of cold stressing was carried out for 7 weeks at a temperature of 1-2°C, starting from 0.5 hour to 6 hours per day by the fourth week of the experiment. The concentration of EE and EA was determined by gas chromatography-mass spectrometry (GC-MS). The analysis of the activity of the ADH and AHD enzymes was performed by standard spectrophotometric methods. The experiment showed that during cold stresses, the survival rate in rats strongly correlates with the EE content in the blood (r=0.757÷0.923). This fact suggests that when adapting cold-adapted animals to the effects of low temperatures, it is important to increase the EE concentration in the body, and the consumption of EE compensates for the increased need of the body in cold conditions for this metabolite. Based on the data obtained, a probable mechanism of the participation of EE in the processes of adaptation of higher vertebrates to cold is presented, which includes specific adaptive changes in the activities of the ADH and AHD enzymes.(**International Journal of Biomedicine. 2019;9(4):356-360.**)

Key Words: adaptation • endogenous ethanol • acetaldehyde • dehydrogenase • laboratory rats • cold stresses

Introduction

The most stressing factor for higher vertebrate organisms in the North is cold. Under these conditions, dehydrogenase enzyme systems, which provide bioenergetic processes even with a lack of oxygen, are very important. One such system includes ADH and AHD metabolizing EE and EA.[1-4] It has been established that maintaining an optimal state of homeostasis when adapting to cold in the series "cold-adapted mammals (including humans) → small cold-adapted animals → large aboriginal cold-adapted animals of the North → hibernating animals," regardless of species specificity and living conditions, is largely due to the ratio of concentrations of EE and EA in the blood, depending on the liver activity of ADH and AHD.[5] It has also been shown that the blood concentration of EE and EA of indigenous people in the conditions of the North is 30%–40% higher than the values of similar parameters in the conditions of the central zone of Russia.[6,7] Moreover, if these homeostatic parameters are stable in humans and animals under conditions of small amplitude of seasonal temperature fluctuations, in the North, people find seasonal dynamics of EE in the blood: in the summer, it is 1.7 times higher than in winter. This, along with the all-season increase in the EA content in the blood, indicates an increase in the importance of these metabolites for life support in extreme climatic conditions.

It is known that EE can be formed when EA is reduced, which occurs in the process of oxidative decarboxylation of pyruvate when the intermediate product is cleaved in the pyruvate decaboxylase complex. It has also been shown that EE can be formed during the decarboxylation of lactate, which accumulates in red skeletal muscles during glycolysis under conditions of oxygen deficiency.[8] In turn, the metabolism of EE and EA is associated with the main metabolism through the formation of acetyl-CoA. The concentrations of EE and EA and their ratio are provided by the enzyme system ADH and AHD. The ADH-dependent reaction is reversible; the AHD-dependent is irreversible (Equation 1). ADH-dependent

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conversion of EE occurs by first oxidizing it to EA, then to acetic acid, followed by the formation of its conjugate with coenzyme A, acetyl-CoA, which further enters the most diverse catabolic and anabolic transformations.

$$EE \underset{ADH2}{\overset{ADH1}{\longleftrightarrow}} EA \overset{AHD}{\longrightarrow} \text{acetic acid} \overset{acetyl\text{-}CoA\text{-}synthase}{\longrightarrow} \text{acetyl CoA} \quad (1)$$

There are 2 groups of AHD isoforms: with high affinity for aldehyde—AHD_1 (Michaelis constant $K_M = 0.1–1.0 \ \mu M$) and with low affinity—AHD_2 ($K_M = 0.1$-1.0 mM).[2,9,10] Due to the heterogeneity of the isoenzyme composition of both enzymes, the levels of EE, EA and their ratios can vary within rather wide limits.

EE and EA are considered to be metabolites that control a large part of the mechanisms of homeostasis, which ensure the optimal vital activity of living organisms, especially under extreme living conditions.[1,5] The normal level of homeostasis of laboratory animals and humans is maintained at blood concentrations of EE of about 0.05-0.2 mM, and EA is about 0.3-0.8 μM. The biological role of EE is diverse: (1) is a high-energy compound and under normal conditions can provide up to 10% of the body's energy needs; (2) participates in the maintenance of the liquid-crystalline, fluid state of the lipid layer of membranes, "liquefying" (fluidizing) them;[11-13] (3) is a regulator of lipid peroxidation in cell membranes, showing properties of a free radical trap and activating cholesterol synthesis.[11]

EA is chemically very active, does not penetrate cell membranes, but can change their permeability to other substances. The biological functions of EA are: (1) regulation of bioenergetic reactions at the stage of terminal oxidation of electron transfer from NADH to FAD by flavin enzymes; (2) regulatory modification (through the formation of Schiff bases) of opioid peptides; (3) with the participation of EA, endogenous morphine and morphine-like compounds are synthesized; (4) regulates the exchange of the most important neurochemical mediators of the amine nature: dopamine, norepinephrine, serotonin; hormone adrenaline.[12-14]

Everything discussed above allows us to consider the EE-EA system as an important element of non-specific regulatory systems of the body, providing the body's adaptive capabilities to the effects of stress, including cold.

Emotional stress, through the above mechanisms, leads to a decrease in the blood levels of EE, then EA, and can provoke their replenishment due to exogenous administration of alcohol.[7,15] Therefore, it has been found that in animals and people with a reduced level of EE in the blood, especially with prolonged exposure of the body to intense-stress environmental factors, an increased tendency to use exogenous ethanol to stabilize homeostasis.

The purpose of this study is to study changes in the state of the system of endogenous ethanol, acetaldehyde and the enzymes that metabolize them during cold stresses in a model experiment, using laboratory animals as an example.

Materials and Methods

Mature male Wistar rats were used in the experiment. Animals in the vivarium were kept in cages at a temperature of 20°-22°C, without limitation of mobility, with free access to water with an adjustable light schedule (12 hours - light, 12 hours - darkness). Simulation of cold stressing was carried out for 7 weeks at a temperature of 1°-2°C, starting from 0.5 hour to 6 hours per day by the fourth week of the experiment. Every day, the animals at the same time were placed in a specially equipped basement room, in which a constant cooling regime of 1°-2°C and a light mode similar to the vivarium were maintained. To maintain the natural rhythm of functioning, the rats were distributed at 4 p.m. At the same time, 2 groups of animals were studied: Group 1 - control (CG) animals were kept in stationary conditions of the vivarium (n=50); Group 2 - experimental (EG) rats were subjected to chronic cold stresses (n=150). After 4 weeks, depending on the nature of the change in the level of EE, the animals of EG were divided into 2 groups. EG1 included animals in which the EE level after the initial decline increased (n=58). EG2 included animals (n=92), in which the EE level decreased, compared with the control. In turn, the animals of EG2 were divided into 2 subgroups: EG2a (n=20) and E2b (n=72). Animals of EG2b, unlike rats of EG2a, were given the opportunity to consume a 10% ethanol solution under conditions of free choice. On Day 50 after the start of the experiment, part of the animals of EG2b (EG2b1; n=20)) were deprived of the possibility of alcohol consumption while maintaining all other conditions. By the end of the 10th week, the survival of the animals in each group was recorded. All blood sampling and decapitations were carried out in the morning when the animals were in a calm state. Blood to determine the concentration of EE and EA was taken from the tail, by cutting the tip of the tail with a sharp razor obliquely spiral. The decapitation of EG1 animals in order to study the ADH and AHD in the liver was carried out on Days 14, 28, and 49 of the experiment.

The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care.

When studying the exchange of EE and EA, the following methods were used:

1. Concentrations of EE and EA in whole blood of animals were determined by GC-MS using a MAESTRO MSD chromatomass-mass spectrometer based on an Agilent 7820 gas chromatograph and an Agilent 5975 mass spectrometer.[17]

2. For kinetic studies, the purification of enzymes from rat liver was performed according to the following procedure. Frozen liver was washed from the blood, repeatedly perfusing it in a chilled saline solution (t=0°C) to a pale-yellow color. The liver tissue was homogenized in the cold. The final dilution of the homogenate was 2:1 (2 ml of a 0.05 M solution of glycine per 1g of the homogenate). To remove incompletely destroyed cells and nuclei, the homogenate was centrifuged for 30 min at 7000g (t=2°C). Chilled ethanol (t=0°C) was added to the supernatant to 20% final concentration. The mixture was shaken vigorously, and then centrifuged again. The supernatant was passed through a column with Sephadex G-100 (1.5×90cm) equilibrated with a 0.5 M glycine solution (t=2°C). In the selected fractions, the activities of ADH and AHD were determined.

3. The activities of ADH and AHD were determined on a Shimazu two-beam spectrophotometer and expressed in µmol/min per 1g of the liver. To obtain the kinetic parameters (Michaelis ADH and AHD constants by NAD, ethanol, NADH, and acetaldehyde), the dependence of the initial velocity on the concentration of one of the substrates (coenzymes) was measured at saturating concentrations of coenzyme. Differentiation of AHD isoforms into 2 groups was performed by the kinetic method.[18]

Statistical analysis was performed using the statistical program Stat Plus 2007. The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean+SEM. Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Mann-Whitney U test test was used to compare means of 2 groups of variables not normally distributed. The frequencies of categorical variables were compared using Pearson χ^2 test. A value of $P<0.05$ was considered significant.

Results and Discussion

Changes in EE concentration detected in the blood of laboratory rats under conditions of low-temperature exposure are presented in Table 1. On Day 14 of cold stress, the EE level decreased by 3.2 times in animals of EG. On Day 28 of the experiment, rats exposed to cold stresses (EG) were divided into 2 groups depending on the level of EE. EG1 included animals whose blood EE level, after the initial decrease, increased by 3.4 times compared to the control, and 5 times on Day 49 of the experiment. EG2 included rats in which the EE level after the initial decrease practically did not increase by Day 28.

Table 1.

The concentration of EE (mmol/l) in the blood of rats during cold stresses

Day	Concentration of EE (mmol/l) in rat blood				P-value
	CG (n=50)	EG (n=150)			
1	0.16±0.03	0.16±0.03			
14	0.17±0.04	0,05±0.01			P=0.0000
		EG1 (n=58)	EG2 (n=92)		
28	0.16±0.04	0.55±0.12	0.08±0.02		$P_{EG1,CG} = 0.0046$ $P_{EG2,CG} = 0.037$ $P_{EG1,EG2} = 0.0059$
		EG2a (n=20)	EG2b (n=72)		
49	0.16±0.03	0.8±0.14	0.07±0.02	1.1±0.21	$P_{EG2a,CG} = 0.0205$ $P_{EG2b,CG} = 0.000$ $P_{EG2a,EG1} = 0.000$ $P_{EG2b,EG1} = 0.1296$ $P_{EG2a,EG2b} = 0.000$

From Day 28 of the experiment, 70 animals of EG2 were given the opportunity to consume a 10% ethanol solution under the conditions of free choice (EG2b), unlike animals of EG2a. On Day 49 after the experiment, the EG2b1 animals were deprived of the possibility of alcohol consumption while maintaining all other conditions. As a result, after 10 weeks, 87% of the animals in this group died (Fig.1). The survival rate of the rats in other groups was as follows: 92% - in EG1, 27% - in EG2a, and 81% - in EG2b.
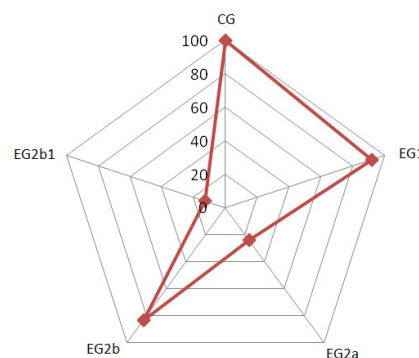
Fig. 1. The survival rate of animals after 10 weeks of the experiment (%).

During cold stressing, the survival rate in rats of all groups strongly correlates with the EE content in the blood (r=0.757÷0.923). This fact suggests that when adapting cold-adapted animals to the effects of low temperatures, it is important to increase the concentration of EE in the body, and the consumption of exogenous ethanol makes up for the increased body need in cold conditions for this metabolite. To identify the mechanisms for increasing the level of EE in the blood during adaptation to cryotherapy, a study was made of the state of the dehydrogenase EE metabolism system in the group of EG1 rats, which have the highest percentage of survival (Table 2).

The kinetic calculation of Scheme (1), carried out considering the obtained concentration values and catalytic parameters of ADH, the activity of AHD_1 and AHD_2, showed that at the first stage of cold stresses (Day 14), a sharp decrease in the concentration of EE is associated with a change in enzyme activities. ADH_1 activity decreases by 1.75 times and ADH_2 activity by 2.1 times, primarily due to a 1.8 times decrease in enzyme concentration. AHD activity also decreases by 1.4 times, first, due to a 1.6 times decrease in the share of AHD_1 (Table 2). Therefore, the EA level is reduced to a lesser extent than EE. By Day 28 of the experiment, there was some restoration of the levels of concentration and activity of ADH. This led to an increase in the EE concentration, in relation to the control, by 3.4 times, and to EA - by 2.0 times. By Day 49 of the experiment, the levels of ADH and AHD completely recovered, which resulted from increasing the concentration of EE by 5 times on the background of increasing the EA level by 5.5 times. This contributed to the smooth flow of cold adaptation.

Table 2.

Characteristics of the metabolic chain of EE in the body of laboratory rats of the EG1 group during cold stressing

Parameters	Control (n = 15)	Cold stress period (days)			P-value
		14 (n=10)	28 (n=10)	49 (n=10)	
$ADH_1^{(1)}$ activity (μmol/min ×g)	0.35±0.04	0.20±0.03	0.25±0.03	0.40±0.04	P_{c-14}=0.0065 P_{14-9}=0.0009 P_{28-49}=0.0080
$ADH_2^{(2)}$ activity (μmol/min ×g)	0.79±0.08	0.37±0.07	0.50±0.08	0.82±0.06	P_{c-14}=0.0006 P_{c-28}=0.0177 P_{14-49}=0.0001 P_{28-49}=0.0052
ADH concentration (nmol/g)	3.1±0.5	1.7±0.4	2.5±0.4	3.3±0.5	P_{c-14}=0.0397 P_{14-49}=0.0230
k_{1ADH} (min^{-1}) $^{(3)}$	113±12	120±12	100±12	120±15	
k_{2ADH} (min^{-2}) $^{(4)}$	255±20	220±20	200±25	248±20	
AHD activity (μmol/min×g), including (%) AHD$_1$ AHD$_2$	1.10±0.12 40±5 60±5	0.80±0.05 25±6 75±6	1.20±0.15 42±5 48±5	1.00±0.09 40±5 60±5	P_{c-14}=0.0308 P_{14-28}=0.0215
[EE], mM	0.16±0.03	0.05±0.01	0.55±0.12	0.8±0.14	P_{c-14}=0.0021 P_{c-28}=0.0046 P_{c-49}=0.0001 P_{14-49}=0.0000 P_{28-49}=0.1928
[EA], μM	1.0±0.2	0.5±0.2	2.0±0.3	5.5±0.3	P_{c-14}=0.0909 P_{c-28}=0.0110 P_{c-49}=0.0000 P_{14-49}=0.0000 P_{28-49}=0.0000
[EE]/[EA]	160±16	100±10	275±28	145±15	

(1) ADH_1 activity in the ethanol oxidation reaction; (2) ADH_2 activity in the acetaldehyde reduction reaction;
(3) The catalytic constant of ADH in the ethanol oxidation reaction; (4) The catalytic constant of ADH in the acetaldehyde reduction reaction.

The mechanism of the cold-adaptive action of EE apparently consists, first, in its ability to easily penetrate into all cells of the body, reduce the viscosity of the lipid layer of cell membranes and optimize functioning at lower temperatures of membrane tissue-specific, transport and receptor complexes. [6,8,9,13] It consists second in the easy and fast mobilization of EE, primarily as a catabolic substrate; therefore, its concentration in the blood at the first stage of adaptation to cold decreases. However, on Day 28 of cold stress, the EE level is not only restored, but increases in overcompensation mode by 3.4-5.0 times. This happens due to specific adaptive changes in the activity of ADH and AHD, as well as to ADH- and AHD-dependent increases in the EA level, which reduces the level of aerobic metabolism and energy losses of the body in cold conditions. [3-5] At the same time, those animals whose EE level remains low on the background of cold stresses (EG2b) begin to feel the need for EE replenishment due to exogenous intake. Realization of this need allows the body to survive during cold stresses. A deprivation of the possibility of exogenous use of ethanol in doses that contribute to cold adaptation leads, with a high probability, to the death of animals (EG2b1). The results of the experiment confirm the significance of ethanol in the processes by which homoiothermal animals adapt to cold.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

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