Effect of Low-Choline Diet and Ethanol on Electroconductivity of Tissues in CBA Mice

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We studied electrophysiological parameters and morphological changes in the adrenal glands and spleen of CBA mice feeding a low-choline diet and/or ethanol-containing diet. The polarization coefficient underwent similar changes in the adrenal glands and spleen, which was associated with different contribution of impedance frequency components. Morphological changes in the adrenal glands were more pronounced than in the spleen and consisted in delipidation of the adrenocortical cell cytoplasm in the zona fasciculata and increase in cell heterogeneity in the zona reticularis of animals of different groups. The observed morphological and electrophysiological changes can serve as a criterion of the severity of stress. The method for a quantitative study of tissue electroconductivity is informative for evaluation of the effects of internal and external factors on organs and tissues.

Key Words: *low-choline diet; ethanol; adrenal glands; spleen; electrophysiology*

Electroconductivity of isolated tissues undergoes specific variations under the influence of contrast temperatures, mechanical injury, hypoxia, and hypokinesia. Moreover, electroconductivity depends on the frequency of electric current [2,11], which is related to polarization and capacitive properties of cells. Little is known about changes in tissue electroconductivity after exposure of the organism to external factors and morphological alterations accompanying these changes. Variations in electrical impedance precede changes in physiological and morphofunctional characteristics of the organism [2,4,11].

Long-term alcohol consumption is a wide-spread exogenous factor that cause serious disorders [3,7-10,12]. Previous experiments showed that feeding a low-choline diet is followed by the development of similar morphological changes in internal organs [13]. The methods for impedance recording can be of considerable prognostic importance, particularly at the stage of reversible morphological changes. A quantitative study of tissues holds promise for evaluation of changes and processes occurring in organs and tissues upon exposure to the effect of internal and external factors.

Here we compared changes in morphological and electrophysiological parameters and polarization properties of the adrenal glands and spleen in CBA mice feeding a low-choline diet and consuming alcohol.

MATERIALS AND METHODS

Experiments were performed on 30 adult male CBA mice weighing 16-18 g. The animals were divided into 3 groups. Group 1 mice fed a low-choline diet and had free access to water. Group 2 mice fed the same diet and received 10% ethanol in 5% glucose as a drinking solution. Group 3 mice received a low-choline diet, ethanol, and thistle seed. Experimental and clinical trials revealed protective activity

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of thistle plants under toxic conditions (*e.g.*, alcohol consumption) [5,7]. Control mice fed a standard diet and had free access to water.

For light microscopy, the left adrenal gland and spleen fragment (3×3×2 mm) were fixed in 4% paraformaldehyde. The samples were treated routinely and embedded in the mixture of Epon and araldite. Semithin sections were obtained on a LKB III ultratome and stained with azure II. PAS reaction was performed.

Electrical characteristics of the adrenal gland and spleen fragment were measured consecutively at low (10 kHz) and high (1 MHz) frequencies (R_{LF} and R_{HF}, respectively) using a Tonus-2 electrical impedancemeter. The polarization coefficient (C_P) was calculated as the ratio between tissue impedances at these frequencies [6]. A needle (tip diameter 60 μ) served as the active electrode. The reference electrode represented a disc, whose area exceeded the maximum size of samples (100 mm^2). The sample was placed on the reference electrode. The active electrode was brought in contact with the sample by rotating a micrometric screw. It was performed to provide electric contact. The total time of measurement for 1 parameter did not exceed 6 sec.

The results were analyzed by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

Two months after the start of the study, C_P was similar for the adrenal gland and spleen from control mice (3.5±0.3). C_P of the adrenal gland and spleen decreased in group 1 (by 16 and 27%, respectively) and 2 mice (by 38%), but approached the control in group 3 animals (Fig. 1).

Changes in C_P of the spleen from group 2 mice were mainly related to a decrease in R_{LF} (30%) and slight increase in R_{HF} (16%). Visually estimated decrease in the volume of the white pulp relative to the volume of the red pulp served as a morphological equivalent of the observed changes. The connective tissue capsule and trabecular fragments were thickened. Cells of the red pulp were characterized by slight perinuclear devastation.

In group 1 mice the values of impedance components were lower compared to the control (by 61 and 84%, respectively). Morphologically, these changes manifested in consolidation and thinning of the connective tissue capsule and trabeculae. Mild edema was detected at the boundary between lymphoid follicles and red pulp.

In group 3 mice the values of impedance components approached the control (Fig. 2). However, these values remained below the control (particularly low-frequency resistance, by 16%). We hypothesized that the observed differences are associated with progression of hemodynamic changes in animals of this group. They were manifested in focal widening and plethora of vessels and central arteries in the red pulp and lymphoid follicles, respectively.

The change in C_P of the adrenal gland from group 2 mice was mainly associated with the increase in R_{HF} (by 56%). The zona fasciculata was well structured and occupied a considerable volume of the adrenal cortex. The cytoplasm of adrenocortical cells in this zone was characterized by diffuse lipid depletion typical of severe stress. We believe that these changes contribute to the decrea-

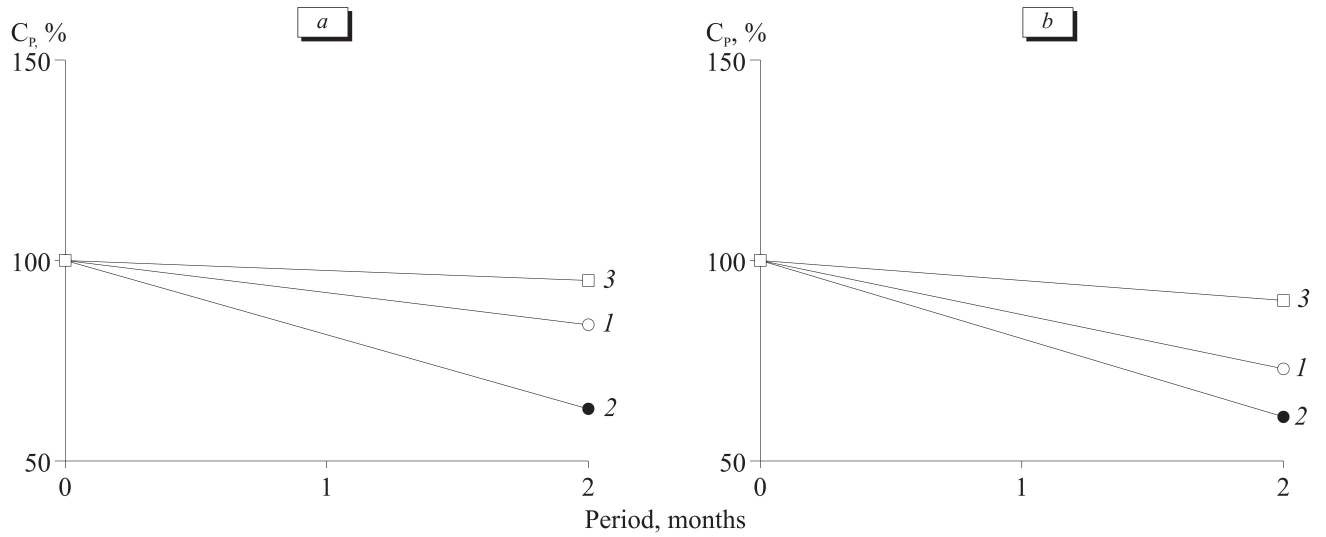

Fig. 1. Polarization coefficient (C_P) of the adrenal glands (*a*) and spleen (*b*) in mice under different dietary regimens. Here and in Fig. 2: groups 1 (*1*), 2 (*2*), and 3 (*3*).

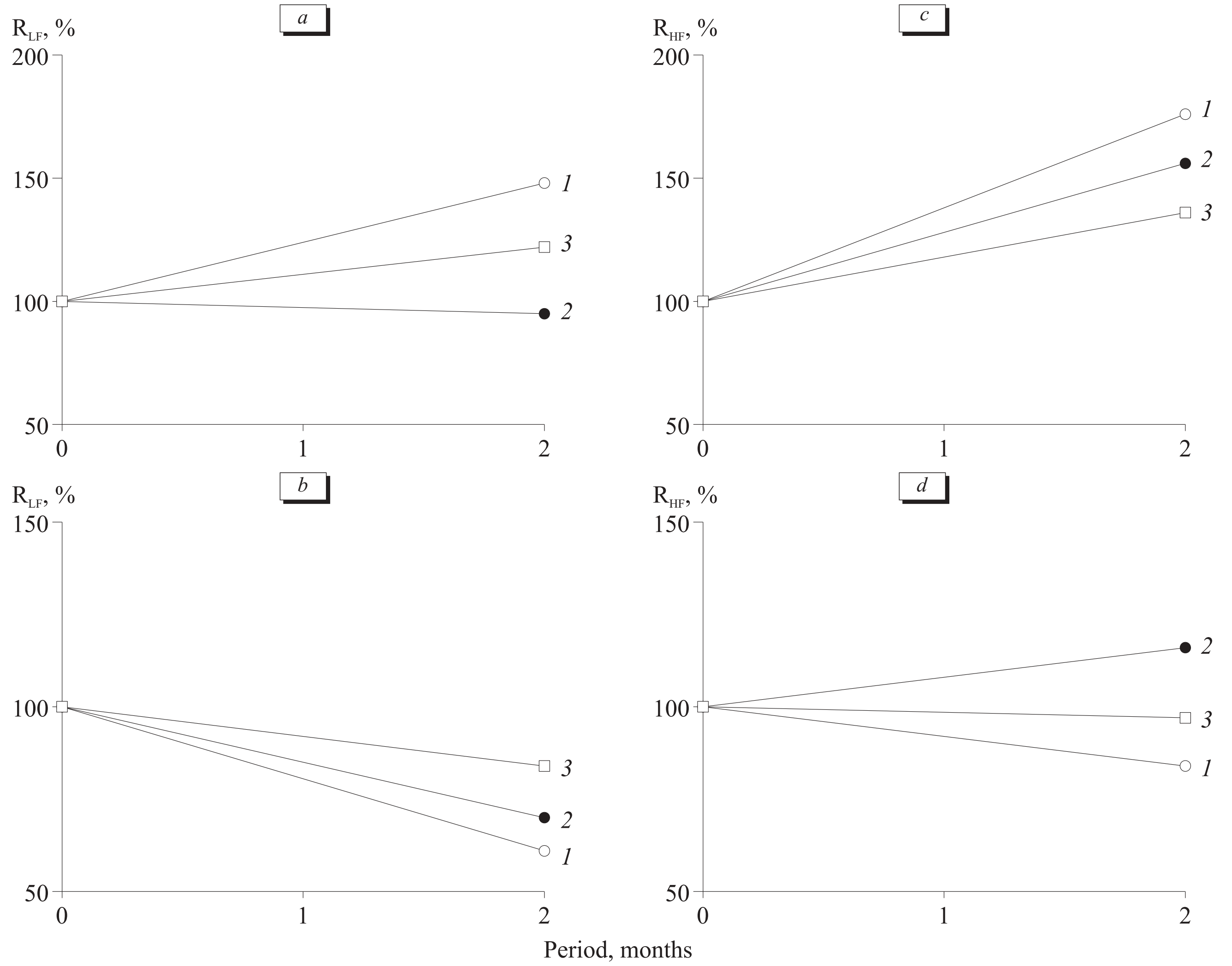

Fig. 2. High-frequency and low-frequency components of impedance (R_{HF} and R_{LF}) in the adrenal glands (*a*, *c*) and spleen (*b*, *d*) of mice under different dietary regimens.

se in C_P. The change in R_{HF} was related to the increase in heterogeneity of cell in the zona reticularis and possible increase in permeability of the cell membrane.

R_{LF} and R_{HF} in group 1 mice were higher than in the control (by 48 and 76%, respectively). The increase in R_{LF} resulted from focal destructive changes in the connective tissue capsule and mild edema of the stroma in the zona glomerulosa. The increase in R_{HF} was associated with a rise in the number of low-vesicular lipid inclusions, which shifted the nuclei to the peripheral region in cells of the outer zona fasciculata. Adrenocortical cells of the inner zona fasciculata contain practically no lipids. These signs are typical of the reaction to mild stress followed by short-lasting strain and stimulation of the adrenal cortex.

In group 3 mice the cytoplasm of adrenocortical cells in the zona fasciculata contained moderate amounts of lipids. The zona reticularis was characterized by high heterogeneity of cells. The cytoplasm of several adrenocortical cells in this zone had large lipid drops. All zones were well structured. This contributed to normalization of R_{LF} and R_{HF}. The state of these animals was described as the adaptive and compensatory reaction. The preparation of thistle plants reduced an adverse effect of alcohol consumption, which was probably associated with the improvement of liver disintoxication function [5,7].

Changes in C_P of the examined organs reflect a general nonspecific reaction to a modified dietary regimen. Intracellular and extracellular structures play a different role in this process. The similarity of changes in CP is associated with opposite variations in R_{LF} and R_{HF}.

We revealed a decrease in the active component (R_{LF}) of impedance in the adrenal glands and spleen of alcohol-consuming animals. The reactive component of impedance (R_{HF}) determined by capacitive properties of the membrane increased in these animals (particularly in the adrenal glands, by 56%). A decrease in C_P of the spleen was mainly associated with hemodynamic changes. These changes in the adrenal glands resulted from the reduction of membrane capacitance due to a decrease in the transmembrane potential and membrane permeability. Feeding a low-choline diet was accompanied by opposite changes in R_{LF} and R_{HF}, which caused a decrease in C_P. High-frequency and low-frequency resistance prevailed in the adrenal gland and spleen, respectively. Consumption of thistle plants produced different changes in R_{HF} and R_{LF} of the adrenal gland and spleen. C_P of examined organs approached the control.

Our results are consistent with published data that stress factors and dietary regimen modulate capacitive properties of the membrane and contribute to changes in the composition of intracellular and extracellular ions [1].

Morphological changes in the adrenal cortex of alcohol-consuming mice reflect the nonspecific stress reaction. It should be emphasized that lipids were partially preserved in animals receiving thistle plants. These data illustrate adaptogenic properties of thistle plants. Structural changes in the spleen were less pronounced than in the adrenal glands. The degree of variations in electrical parameters did not differ in the adrenal glands and spleen, *i.e.* occurred in the earlier period. Variations in C_P and morphological changes in the adrenal glands can serve as a criterion for the severity of stress.

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