Effect of Citrocard on Functional Activity of Cardiomyocyte Mitochondria during Chronic Alcohol Intoxication

V. N. Perfilova, O. V. Ostrovskii, V. E. Verovskii, T. A. Popova, S. A. Lebedeva, and H. Dib

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 3, pp. 312-314, March, 2007
Original article submitted October 4, 2006

Chronic administration of 50% ethanol in a dose of 8 g/kg produces a toxic effect on functional activity of cardiomyocyte mitochondria, which manifested in decreased rates of respiration and oxidative phosphorylation. Structural GABA analogue Citrocard (phenibut citrate) and reference preparation piracetam in doses of 50 and 200 mg/kg, respectively, prevented the damaging effect of alcohol, which was seen from increased indexes of oxidative phosphorylation in treated animals compared to the control group.

Key Words: *alcohol intoxication; mitochondria; oxidative phosphorylation*

Activation of lipid peroxidation [1,3] and dysfunction of the oxidative phosphorylation system [4,5] play an important role in the pathogenesis of myocardial injury under the influence of ethanol. Ethanol-induced suppression of the antioxidant system leading to impairment of mitochondrial structure [1,3], formation of ethyl esters of fatty acids blocking their oxidation, and metabolic dysfunction in the myocardium accompanied by inhibition of NAD-dependent dehydrogenases, electron transport chain enzymes, and ATP synthesis contribute to energy deficiency in cardiomyocytes and decrease the effectiveness of energy consumption by myocardial structures [4,5].

GABA and its analogues stimulate respiration, oxidative phosphorylation, and metabolism of the major energy sources [2]. Here we studied the effect of GABA derivative Citrocard (phenibut citrate) and reference preparation piracetam on oxidative and phosphorylation functions of mitochondria under conditions of chronic alcohol intoxication (CAI).

Department of Pharmacology and Biopharmacy, Faculty of Postgraduate Medical Training, Institute of Pharmacology, Volgograd State Medical University

MATERIALS AND METHODS

Experiments were performed on 50 male outbred albino rats weighing 210-240 g. CAI was induced by intragastric administration of 50% ethanol in a daily dose of 8 g/kg for 30 days. Group 1 animals (positive control) intragastrically received an equivalent volume of physiological saline for 30 days. Group 2 alcoholized animals (negative control) intraperitoneally received physiological saline in a volume equivalent to that of the test preparations. Citrocard (group 3) and piracetam (group 4) in doses of 50 and 200 mg/kg, respectively, were injected intraperitoneally 40 min before ethanol administration.

The animals were cold-euthanized by the end of alcoholization. The hearts were removed. Myocardial mitochondria were isolated by differential centrifugation. Oxidation and phosphorylation in mitochondria were studied polarographically in an incubation medium containing 0.3 M sucrose, 0.01 M KCl, 0.005 M potassium phosphate, and 0.001 M EDTA (pH 7.4). NADH (0.001 M) served as the oxidation substrate. ADP was added to a final concentration of 200 μM. Protein content was measured by the method of Bradford. The following parameters were recorded: V_1, rate of O_2 consump-

tion by mitochondria in the absence of substrate (mg-atom/mg protein/min); V_3, rate of O_2 consumption after ADP addition; V_4, rate of O_2 consumption after ADP depletion; CRC, Chance respiratory control (V_3/V_4); and ADP/O, index of phosphorylation efficiency.

The significance of differences between mean values in control and treatment groups was evaluated by Student's *t* test with Bonferroni correction.

RESULTS

Mitochondrial function was suppressed in alcoholized animals, which was seen from the decrease in V_1, V_4, and V_3 by 29.4, 34.9, and 56%, respec-

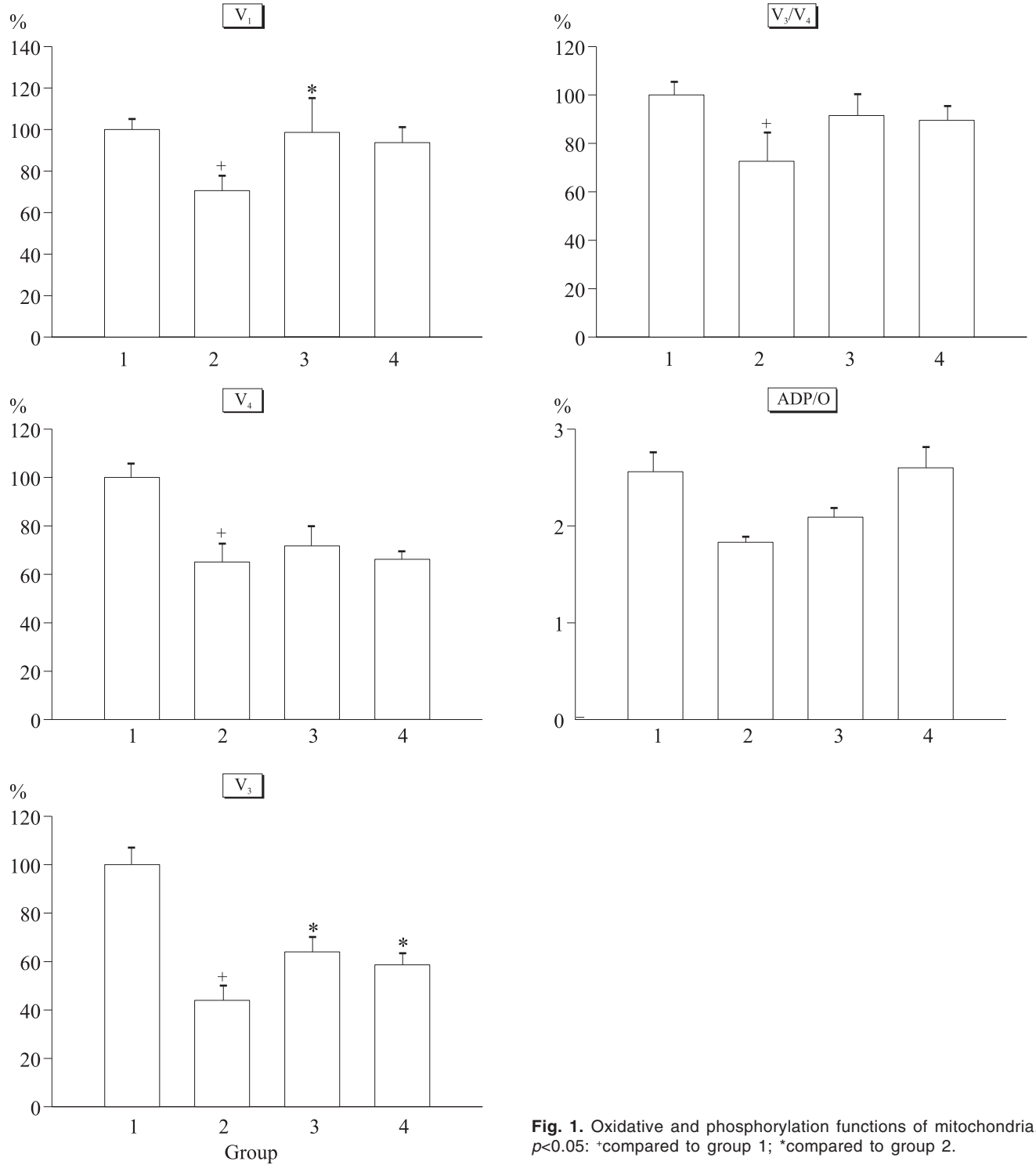

Fig. 1. Oxidative and phosphorylation functions of mitochondria. $p<0.05$: +compared to group 1; *compared to group 2.

tively, compared to intact animals (Fig. 1). CRC and ADP/O decreased by 27.4% and 1.4 times, respectively.

Dysfunction of respiration and oxidative phosphorylation was less severe in group 3 rats. V_1 in group 3 animals was 39.6% higher than in group 2 rats. V_4 in group 3 rats was 28.3% lower than in group 1 animals, but 10.1% higher compared to group 2 rats. V_3 in group 3 rats was 36% lower than in group 1 animals, but 45.4% higher compared to group 2 rats ($p \leq 0.05$). CRC in group 3 rats was 9.2% lower than in group 1 animals, but 26% higher compared to group 2 rats (Fig. 1). ADP/O in group 3 rats did not differ from that in group 1 animals, but was 1.4-fold higher compared to group 2 rats (Fig. 1).

V_1 in group 4 rats was 32.7% higher than in group 2 animals ($p \leq 0.05$). V_4 in group 4 rats decreased by 33.8% compared to group 1 animals, but did not differ from that in group 2 rats. V_3 in group 4 rats was 42% lower than in group 1 animals ($p \leq 0.05$), but 31.8% higher compared to group 2 rats (Fig. 1). CRC in group 4 rats was 23.3% higher than in group 2 animals. ADP/O did not differ in rats of groups 4 and 2 (Fig. 1).

Our results show that ethanol decreases the rate of O_2 consumption by mitochondria, inhibits energy formation, suppresses electron transport in the respiratory chain, and impairs oxidation-phosphorylation coupling. The inhibition of phosphorylation is more pronounced than respiratory dysfunction. This conclusion is derived from the fact that V_3 and V_4 in treated rats decrease by 56 and 34.9%, respectively, compared to group 1 animals.

Citocard and piracetam prevent the damaging effect of ethanol on mitochondrial energy production. These preparations abolish the decrease in the rate of O_2 consumption by mitochondria and inhibition of oxidative phosphorylation, which was seen from the increase in V_1, V_3, CRC, and ADP/O in rats receiving the test preparations compared to animals receiving ethanol.

REFERENCES

1. I. V. Koshkin and T. A. Bukach, *Alcohol Injury to the Heart* [in Russian], Naberezhnye Chelny (2001).
2. M. D. Mashkovskii, *Medicinal Preparations* [in Russian], Moscow (2005).
3. S. V. Okovityi, *Clinical Pharmacology of Antioxidants* [in Russian], FARMindeks-Praktik (2003), Vyp. 5, pp. 85-111.
4. J. Kauhanen, G. F. Kaplan, D. E. Goldberg, *et al*., *Arterioscler. Thromb. Vasc. Biol.*, **19**, No. 2, 3001-3006 (1999).
5. Z. Keatsky Arthaus, *Alcohol in Health and Disease*, New York (2001), pp. 517-546.