AUCTORES
Globalize your Research

Biotechnology and Bioprocessing
E. I. Bon *

Open Access

Research Article

State of Tissue Respiration of Brain Regions in Chronically Alcoholic Rats Exposed to Ethanol and Succinate in in Vitro

V. Lelevich, E. I. Bon *, T. I. Ilyuchik, E. A.Yankovskaya

Grodno State Medical University, Grodno, Belarus.

***Corresponding Author:** E. I. Bon, Grodno State Medical University, Grodno, Belarus.

Received date: March 05, 2025; **Accepted date:** March 10, 2025; **Published date:** March 16, 2025

Citation: V. Lelevich, E. I. Bon, T. I. Ilyuchik, E. A.Yankovskaya, (2025), State of Tissue Respiration of Brain Regions in Chronically Alcoholic Rats Exposed to Ethanol and Succinate in in Vitro, *J, Biotechnology and Bioprocessing*, 6(2); **DOI:10.31579/2766-2314/152**

Abstract

The problem of the development of alcohol dependence remains relevant due to insufficient research on the processes occurring in the brain during prolonged exposure to ethanol. The aim of the study is to assess the rate of oxygen consumption by homogenates of the cerebral cortex and cerebellum of rats under conditions of chronic alcoholization, ethanol withdrawal, as well as the influence of ethanol and succinate in vitro on it. The rate of oxygen consumption by homogenates of the cerebral cortex and cerebellum of rats receiving ethanol for 8 months was studied, as well as during the period of ethanol withdrawal on endogenous substrates, during incubation with a solution of ethanol and succinate. An increase in the rate of oxygen consumption by brain homogenates on endogenous substrates was found during chronic alcohol intoxication of rats, a decrease during the withdrawal of ethanol on the 1st and 3rd days, a stimulating effect of ethanol in the cerebral cortex on the 3rd day of abstinence, as well as a stimulating effect of succinate in the groups of control animals and with chronic alcohol intoxication. Thus, chronic alcoholization of rats leads to the development of dependence of tissue respiration on the presence of ethanol in the cell. The absence of a stimulating effect of succinate in the groups with ethanol withdrawal indicates significant activation of the succinate dehydrogenase pathway in these animals.

Key Words: chronic alcohol intoxication; alcohol withdrawal; rats; tissue respiration; brain homogenates

Introduction

Alcohol has an adverse effect not only on the health of an individual, but also on social and demographic processes in society [1]. Prolonged intake of ethanol in the body leads to the formation of physical dependence, to which there is a hereditary predisposition [2]. Up to half of people with long-term alcohol use experience a withdrawal state where consumption is significantly reduced or stopped. In the most severe form, it can be life-threatening [3]. However, its pathogenesis requires further investigation [4].

According to some authors, the manifestation of clinical signs of alcohol withdrawal syndrome is based on disorders of metabolic processes in the mitochondria [5]. Chronic alcohol consumption in experimental animals causes hypoxia in various organs [6,7]. It has been shown that brief cyclical episodes of moderate hypoxia and reoxygenation in experimental animals for several days or weeks cause adaptation that protects the brain from glutamate excitotoxicity caused by ethanol withdrawal, mitochondrial damage, reduced ATP synthesis, oxidative stress, accumulation of beta-amyloid [8], and protects rat cerebellar mitochondrial cytochrome c oxidase from the stress associated with ethanol withdrawal [9], reduces alcohol consumption and withdrawal symptoms [10]. In the pathogenesis of withdrawal syndrome, the functional and biochemical aspects of this condition are the least studied. There is little literature evidence that both acute alcoholism and chronic alcoholism are accompanied by metabolic shifts in the mitochondria of nerve cells, which leads to a decrease in the intensity of energy-producing processes.

Thus, the processes that occur in brain cells during chronic alcohol intoxication (CAI) and abstinence require further study, the question remains unclear: does ethanol itself affect the cells or is it the effect of more toxic acetaldehyde?

The aim of the study was to evaluate the rate of oxygen consumption by homogenates of the cerebral cortex and cerebellum of rats under conditions of chronic alcoholism, ethanol withdrawal, as well as the effect of ethanol and succinate in on it in vitro.

Materials and methods

Experiments were performed on 28 white outbred male rats. The experiments were conducted in accordance with the principles of experimental and clinical bioethics. The weight of the animals was 160-230 g.

CAI was modeled by the method of incomplete water deprivation [11]. The experimental group of rats consumed an ethanol solution as the only source of liquid for 8 months. The concentration of the ethanol solution during the

first 2 weeks was 5%, the next 2 weeks-10%, and then until the end of the experiment – 15%. The animals were kept on dry food. The control group was kept in similar conditions and consumed water.

Alcohol withdrawal syndrome was modeled in chronically alcoholic rats (8 months) by replacing the ethanol solution with water for periods of time equal to the 1st and 3rd days.

The following experimental groups were formed: 1st-control (n=7), 2nd-CAI (n=7), 3rd – 1st day of ethanol withdrawal (n=7), 4th-3rd day of ethanol withdrawal (n=7).

After decapitation, the animals ' brains were quickly removed from them, cleaned of blood, and холодethe cerebral cortex and cerebellum were isolated in the cold. Homogenates were prepared.

Tissue respiration was determined by the rate of oxygen consumption (SPC) by rat brain homogenates. The procedure was performed in п polarographicclosed temperature-controlled cell with a volume of 1.25 ml using a Clark electrode [12]. During the experiment, after recording the initial oxygen consumption (respiration on endogenous substrates), an ethanol solution in the final concentration of 50 mmol/l was added to the medium and changes in O2 consumption were recorded$_2$. After that, 5 mmol/l of sodium succinate was added to the cell and the oxygen consumption stimulated by it was recorded.

The results were expressed as median (Me) and scattering (25, 75 percentiles). Nonparametric criteria and Kruskal-Wallis, U-Mann-Whitney, and TWilcoxon T-test were used to compare the values Вилкоксона. The differences were considered statistically significant at $p<0.05$. Statistical data processing was performed using the STATISTICA 10.0 package.

Results and discussion

In the homogenates of the cerebral cortex of rats with CAI, an increase in SPC was observed in comparison with the control group by 83.3%, p=0.02 (Table 1).

	Endogenous	Ethanol (50 mol/l)	Succinate (5 mmol / l)
Control (n=8)	0,006 (0,0046; 0,009)	0,0059 (0,0026; 0,0078)	0,024 > (0,02; 0,027)
KHAI (n=7)	0,011 * (0,0087; 0,014)	0,014 (0,007; 0,021)	0,029 > (0,025; 0,036)
1 day of ethanol withdrawal (n=7)	0,0036 + (0,0013; 0,0065)	0,011 (0,0087; 0,016)	0,018 (0,005; 0,026)
3 days of ethanol withdrawal (n=6)	0,009+ (0,003; 0,009)	0,013# (0,0096; 0,014)	0,011 (0,0076; 0,012)

Table 1: Rate of oxygen consumption by homogenates of the cerebral cortex of chronically alcoholic rats duringin in vitro incubation with ethanol solution and succinate, Iu (25 %; 75 %), ml O_2 x min / g of tissue

Table 1. The rate of oxygen consumption by homogenates of the cerebral cortex of chronically alcoholized rats during in vitro incubation with ethanol solution and succinate, Me (25%; 75%), ml O_2 x min / g tissue

Notes:

1 - * - statistically significant differences with the control group,

2 - + - with the KHAI group,

3 - # – with the same group, respiration on endogenous substrates,

4 - > – with the same group, incubation with ethanol solution

Withdrawal of ethanol on day 1 causes a decrease in SPC in rats compared to CAI by 67.3%, p=0.0027, and withdrawal of ethanol on day 3-by 18.2%, p=0.045.

To study the direct effects of ethanol, experiments were conducted with its addition in in vitro. It was found that ethanol increases SPC in the group of rats with ethanol withdrawal on day 3 by 44.4%, p=0.04, compared with endogenous respiration in the same group of rats. After incubation of homogenates with ethanol, stimulation of respiration with succinate leads to an increase in SPC by homogenates of the cerebral cortex of rats in control animals by 306.8% (p=0.01) and in the group with CAI – by 107.1% (p=0.01). No stimulating effect of succinate was found in the groups of rats with ethanol withdrawal.

Similar results were obtained when studying tissue respiration in cerebellar homogenates. So, in CAI, there is an increase in SEC compared to the control group by 225.0 %, p=0.002 (Table 2).

	Endogenous	Ethanol (50 mol/l)	Succinate (5 mmol / l)
Control (n=8)	0,004 (0,003; 0,0057)	0,0052 0,0052(0,004; 0,0069)	0,021> (0,014; 0,028)
KHAI (n=7)	0,013* (0,011; 0,018)	0,017 (0,0076; 0,024)	0,031> (0,024; 0,044)
1 day of ethanol withdrawal (n=7)	0,0028+ (0,0015; 0,0047)	0,007 (0,0032; 0,015)	0,021 (0,02; 0,025)
3 days of ethanol withdrawal (n=6)	0,0018*+ (0,0014; 0,0023)	0,013 (0,0061; 0,024)	0,021 (0,015; 0,023)

Table 2: Rate of oxygen consumption гомогенатамиву cerebellar homogenates of the brain of chronically alcoholized rats duringin in vitro incubation with ethanol solution and succinate, Iu (25 %; 75 %), ml O_2 x min / g of tissue

Table 2. The rate of oxygen consumption by cerebellar homogenates of the brain of chronically alcoholized rats during in vitro incubation with ethanol solution and succinate, Me (25%; 75%), ml O_2 x min / g tissue

Notes:

1 - * - statistically significant differences with the control group,

2 - + - with the KHAI group,

3 - > – with the same group, incubation with ethanol solution

Withdrawal of ethanol in rats on day 1 causes a decrease in SPC compared to CAI by 78.5%, p=0.001, on day 3 of withdrawal-by 86.2%, p=0.006. On

day 3 of ethanol withdrawal, SPC decreases by 55.0% compared to the control group, p=0.049.

However, the addition of ethanol to the cerebellar homogenates did not lead to changes in the studied indicator.

When cerebellar homogenates were stimulated to breathe with succinate, an increase in SPC was also observed only in the control group by 303.8%, p=0.02, and in the group of rats with CAI by 82.4%, p=0.01. In both groups of rats with ethanol withdrawal, the stimulating effect of succinate was absent.

It can be assumed that in CAI, an increase in SPC reflects an increased need for oxygen in the nervous tissue, which indicates the existence of adaptive mechanisms that arise in response to prolonged alcohol intake in the body. This adaptive mechanism allows efficient functioning of the processes of tissue respiration, ensuring the oxygen demand of the brain in alcoholic rats. The decrease in the rate of oxygen consumption after ethanol withdrawal may be due to a decrease in the activity of tissue respiration enzymes, which is confirmed by literature data indicating a decrease in the activity of cytochrome oxidase in the cerebral cortex at 6 months of alcoholization of animals and a decrease in the activity of succinate dehydrogenase at the 12th month of alcoholization [13]. Our previous studies showed a decrease in the intensity of NADH-dependent oxidation during abstinence in rats [14].

An increase in SPC with the addition of ethanol in гомогенатахrat cortical homogenates with the elimination of ethanol on day 3 indicates that ethanol itself is a factor in increasing SPC. According to Komissarova I. A., it is the drop in the concentration of acetaldehyde after ethanol withdrawal in long-term alcoholized rats that is the main factor leading to a decrease in the activity of $NADH2_2$-dependent dehydrogenases, changes in metabolic processes in general, and leads to the development of alcohol withdrawal syndrome [15]. However, our data suggest that ethanol itself can directly affect the rate of oxygen consumption гомогенатамиby rat brain homogenates and, most likely, activate tissue enzymes. Apparently, ethanol was an activator of tissue respiration when added to гомогенатамbrain homogenates of rats with alcohol withdrawal.

Inhibition of the respiration rate after ethanol withdrawal and its pronounced increase during incubation of homogenates with ethanol indicate the need for the presence of ethanol in an increased concentration for the processes of tissue respiration in alcoholic rats. The absence of a stimulating effect of succinate in groups of animals with ethanol withdrawal and literature data indicate an increase in the activity of succinate dehydrogenase with alcohol withdrawal in rats.

Conclusions

During chronic alcohol intoxication in rats, an increase in the respiratory activity of homogenates of the cerebral cortex on endogenous substrates occurs.

During the period of ethanol withdrawal, oxygen utilization by rat brain homogenates is disrupted, and the rate of oxygen consumption in the cerebral cortex and cerebellum decreases on the 1st and 3rd days of abstinence.

Ethanol in in vitro does not change the rate of oxygen consumption by rat brain homogenates during chronic alcohol intoxication. In alcohol withdrawal, ethanol stimulates the rate of oxygen consumption in the cerebral cortex on the 3rd day of ethanol withdrawal.

The addition of succinate to brain homogenates increases the rate of oxygen consumption in groups of control animals and those with chronic alcohol intoxication. The absence of a stimulating effect of succinate in the groups with ethanol withdrawal indicates a significant activation of the succinate dehydrogenase pathway in these animals.

References:

1. The Global Impact of Alcohol Consumption on Premature Mortality and Health in 2016 / I. Sohi [et al.] (2021). // Nutrients. (13), 9.
2. Searching for an environmental effect of parental alcoholism on offspring alcohol use disorder: a genetically informed study of children of alcoholics / W. S. Slutske [et al.] // J Abnorm Hsychology. – 2008. – Vol. 117, № 3. – P. 534-551.
3. Tiglao, S. M. Alcohol withdrawal syndrome: outpatient management / S. M. Tiglao, E. S. Meisenheimer, R. C. Oh // Am Fam Physician. – 2021. – Vol. 104, iss. 3. – P. 253-262.
4. Research Needs for Inpatient Management of Severe Alcohol Withdrawal Syndrome: An Official American Thoracic Society Research Statement / T. L. Steel [et al.] // Am J Respir Crit Care Med. – 2021. – Vol. 204, iss. 7. – P. e61-e87.
5. Komissarova I. A. Biochemicheskie osnovy formirovaniya alcoholizma i problemy metabolicheskoi terapii [Biochemical bases of alcoholism formation and problems алкоголизма: of metabolic therapy]. Моск Research Institute of Psychiatry; EditorialBoard: V. V. Kovalev [et al.], Moscow, 1988, pp. 70-74.
6. Regulation of heme oxygenase expression by alcohol, hypoxia and oxidative stress / L. N. Gerjevic [et al.] // World J Biol Chem. – 2011. – Vol. 2, iss. 12. – P. 252-260.
7. Nitric oxide and hypoxia exacerbate alcohol-induced mitochondrial dysfunction in hepatocytes / B. R. Zelickson [et al.] // Biochim Biophys Acta. – 2011. – Vol. 1807, iss. 12. – P. 1573-1582.
8. Jung, M. E. Intermittent hypoxia training: Powerful, non-invasive cerebroprotection against ethanol withdrawal excitotoxicity / M. E. Jung, R. T. Mallet // Respir Physiol Neurobiol. – 2018. – Vol. 256. – P. 67-78.
9. Intermittent hypoxia conditioning protects mitochondrial cytochrome c oxidase of rat cerebellum from ethanol withdrawal stress / X. Ju [et al.] // J Appl Physiol. – 2012. – Vol. 112, iss. 10. – P. 1706-1714.
10. Adaptation to periodic hypoxia reduces the consumption of ethanol and withdrawal injuries of internal organs when it is canceled in chronically alcoholic animals / F. Z. Meerson [et al.] / / Bulletin of Experimental Biology and Medicine. - 1992. - Vol. 114, No. 12. - pp. 574-578.
11. Burov Yu. V., Zhukov V. N. Biologicheskie modeli khronicheskogo alcoholizma [Biological models of chronic alcoholism]. Teoreticheskie osnovy poisk sredstv dlya lechenii alcoholizma: obzoryzy [Theoretical foundations of the search for drugs for the treatment of alcoholism: reviews]. Moscow: VINITI Publ., 1984, pp. 57-92.
12. Kovalenko E. А.Полярографическое, Berezovsky V. A., Epshtein I. M. Polarographic determination of oxygen in the body. Moscow: Meditsina Publ., 1975, 232 p. (in Russian)
13. BelokrinitskiyV. S., Mironets N. V., Martynenko N. V. Vliyanie malykh kontsentratsiy alcoholok na aktivnost ' okislitel'no-vosstanovitel'nykh fermentov mozgovogo mozga [Influence of small alcohol concentrations on the activity of redox enzymes of the brain]. Laboratornoe delo, 1982, no. 11, pp. 113-115.
14. Lelevich A.V., Lelevich S. V. Violations of metabolism during the introduction of ethanol into the body: monograph / A.V. Lelevich, S. V. Lelevich. – Grodno: GrSMU, 2017. - 132 p.
15. Komissarova I. A. Mekhanizmy formirovaniya alcoholizma [Mechanisms of alcoholism formation] // Questions of narcology. - 1994. - No. 4. - pp. 19-22.

DOI:10.31579/2766-2314/152