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Respiration in isolated cardiomyocytes and microviscosity of their membranes in rats of different ages with heart failure

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ABSTRACT

Background. According to the latest epidemiological data, heart failure (HF) is diagnosed in 10% of adult population over 70 years old. However, currently this diagnosis is being increasingly made in young and middle-aged people. The pathogenesis of HF may be based on a decrease in respiration in cardiomyocytes with age, which affects the function of energy-dependent processes in cells.

Aim. To study respiration in cardiomyocytes and microviscosity of their membranes in rats aged 2 and 15 months with heart failure.

Materials and methods. The study was carried out on male Wistar rats. The animals were divided into 4 groups: 2 groups of intact rats aged 2 and 15 months (n = 12) and 2 groups of animals of similar ages (n = 10) with a model of HF. In the latter, HF formed by day 28 after a double subcutaneous injection of isoproterenol hydrochloride at a dose of 170 mg / kg with an interval of 24 hours. Isolated cardiomyocytes were obtained from enzyme-washed rat hearts. Cell respiration was studied in a thermostated chamber in an incubation medium supplemented with phosphorylation (ADP) and oxidation (succinate) substrates. The respiratory control (RC) ratio was calculated by dividing V3 respiration state to V4. Membrane microviscosity characteristics were assessed by the eximerization coefficient of pyrene fluorescence in the areas of protein – lipid and lipid – lipid interactions.

Results. RC in cardiomyocyte membranes of intact animals did not change with age. In 2-month-old rats with HF, respiratory control ratio (RCR) did not change compared with the intact age-matched controls. In 15-month-old rats with HF, there was a significant decrease in RC of cardiomyocytes (CM) compared with the intact animals of this age and 2-month-old rats with HF. An age-dependent decrease in the microviscosity of CM membranes in the areas of lipid – lipid interactions and no significant changes in the parameter at the sites of protein – lipid interactions were noted. In 2-month-old animals with HF, the microviscosity of CM membranes in the areas of protein – lipid interactions significantly decreased, and in 15-month-old rats it increased, compared with the intact controls. When carrying out an intergroup comparison, an age-dependent increase in the microviscosity of CM membranes in the areas of protein – lipid interactions and no differences in the parameter in the areas of lipid – lipid interactions were revealed.

Conclusion. In the intact rats, the absence of significant changes in respiration with age was revealed. In the 15-month-old animals with HF, respiration in CM was significantly lower than in the intact controls and 2-month-old animals with HF. These changes may be due to the differences in the membrane microviscosity characteristics in different periods of ontogenesis.

Keywords: cardiomyocyte respiration, membrane microviscosity, age-dependent changes, rats

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Активность дыхания изолированных кардиомиоцитов и микровязкость их мембран у крыс разных возрастов при сердечной недостаточности

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РЕЗЮМЕ

Актуальность. По последним эпидемиологическим данным, сердечная недостаточность (СН) диагностирована у 10% взрослого населения старше 70 лет, при этом все чаще данный диагноз ставят лицам молодого и среднего возраста. В основе патогенеза СН может лежать снижение дыхания в кардиомиоцитах с возрастом, что отражается на работе энергозависимых процессов в клетках.

Цель. Изучить дыхательную активность и микровязкость мембран кардиомиоцитов (КМ) крыс в возрасте 2 и 15 мес при сердечной недостаточности.

Материалы и методы. Исследование проведено на самцах крыс линии Wistar. Сформировано четыре группы животных: две группы интактных крыс в возрасте 2 и 15 мес (n = 12) и две группы животных аналогичных возрастов (n = 10), у которых моделировали развитие СН, формировавшуюся к 28-м сут после двукратного подкожного введения изадрина гидрохлорида в дозе 170 мг/кг с интервалом 24 ч. Изолированные кардиомиоциты получали из промытого ферментами сердца. Изучение активности дыхания клеток проводили в термостатируемой камере в инкубационной среде с добавлением субстратов фосфорилирования (АДФ) и окисления (сукцинат). Рассчитывали коэффициент дыхательного контроля (ДК) как отношение скоростей убыли кислорода в метаболических состояниях V3 и V4. Микровязкостные характеристики мембран оценивали по коэффициенту эксимеризации флуоресцентного зонда пирен в зонах белок-липидных и липид-липидных контактов.

Результаты. Дыхательный контроль в КМ интактных животных с возрастом не претерпевал изменений. При СН у 2-месячных крыс ДК не изменялся относительно интактного возрастного контроля. У 15-месячных крыс с СН происходит значимое снижение ДК в КМ как относительно интактных животных этого возраста, так и относительно 2-месячных крыс с СН. Отмечено возраст-зависимое снижение микровязкости мембран КМ в зонах липид-липидных взаимодействий без значимых изменений показателя в местах белок-липидных контактов. При СН у 2-месячных животных микровязкость мембран КМ в зонах белок-липидных взаимодействий существенно понижается, а у 15-месячных повышается относительно интактного контроля в группе. Межгрупповое сравнение выявило возраст-зависимое увеличение микровязкости мембран КМ в области белок-липидных контактов и отсутствие различий в фазе общих липидов.

Заключение. Выявлено отсутствие значимых изменений дыхания с возрастом у интактных крыс. При СН у 15-месячных животных дыхание КМ значительно ниже интактного контроля и 2-месячных животных с СН. Данные изменения могут быть обусловлены различием микровязкостных характеристик мембран клеток в разные периоды онтогенеза.

Ключевые слова: дыхание кардиомиоцитов, микровязкость мембран, возраст-зависимые изменения, крысы

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Heart failure (HF) is a common outcome in a multitude of cardiovascular diseases. According to epidemiological studies, with age, the incidence of HF increases from 3% in the age group of 46–64 years to 10% in the age group of 70 years and older [1]. Recently, a rising incidence of HF in younger adults has been noted. The results of European studies have shown an increase in the proportion of patients under 50 years with new-onset HF [2, 3]. A decrease in respiration in cardiomyocytes (CMs) at later stages of ontogenesis may be one of the possible pathogenetic factors in the development of HF [4]. It is known that the state of a lipid bilayer in biological membranes affects their functioning and the functioning of membrane-bound enzymes [5].

The aim of the study was to compare respiration in CMs and microviscosity characteristics of their membranes in rats of different age groups with HF.

MATERIALS AND METHODS

The research was performed on male Wistar white rats. The animals were divided into 4 groups: 2 groups of intact rats aged 2 and 15 months (n = 12) and 2 groups of animals of similar ages (n = 10) with a model of HF. The isoproterenol-induced HF model was used in the study. Two subcutaneous injections of isoproterenol hydrochloride (Isadrin, Sigma, USA) with an interval of 24 hours (170 mg / kg of rat body weight) were performed. HF developed by day 28 after the second injection [6]. By the time the groups were formed, the weight of 2- and 15-month-old rats averaged 199 (198; 203) and 528 (500; 563) g, respectively. All manipulations on the rats were carried out in compliance with the provisions of the order of the Ministry of Health of the Russian Federation of April 1, 2016 No. 199n "On Approval of the Rules of Good Laboratory Practice". The study was performed within the fundamental research topic of the Cardiology Research Institute, Tomsk NRMC AAAA-A15-115123110026-3. The study was approved by the Bioethics Committee at the Cardiology Research Institute, Tomsk NRMC (Protocol No. 192 of 18.12.2019).

CMs were separated from the isolated heart. The anesthetized rat was submitted to thoracotomy, which provided access to the heart. The excised heart was transferred into ice-cold Krebs – Henseleit solution (mM) (NaCl – 118;KCl – 4.7; KH_2PO_4 – 1.25; $MgSO_4$ – 1.3; $CaCl_2$ – 1.2; glucose – 10) (pH = 7.35–7.40) (Sigma, USA). The connective tissue was removed, isolating the aorta; and the organ was washed from blood. Then it was transferred to the perfusion chamber and perfused via the aorta with oxygenated (95% O_2, 5% CO_2) Krebs – Henseleit solutions with different content of Ca^{2+} ions and proteolytic enzymes (collagenase type II (PanEco, Russia) – 0.2 mg / ml and pronase (Roche Diagnostics, USA) – 0.1 mg / ml). At the end of the perfusion procedure, the enzyme-washed heart was placed in Krebs – Henseleit solution, the ascending aorta was removed, and the heart was cut into 1–2 mm^3 pieces. Then a suspension of isolated CMs was obtained by gentle pipetting [4].

The concentration of total protein in the obtained cell suspension was determined by the micro Lowry assay using a set of Sigma reagents (USA). The optical density in the studied samples was measured by the NanoDrop spectrophotometer (USA) against a reagent blank at λ = 630 nm.

CM respiration was measured on the Expert – 001 fluid analyzer (Ekoniks, Russia) using the Clark DKTP-02.4 sensor immediately after the isolation. An aliquot of cell suspension (protein content of 0.5–1 mg) was placed in the thermostated (t = 25–27 ºC) 1 ml chamber with a pre-oxygenated incubation medium (mM) (sucrose – 0.25; KCl – 10; KH_2PO_4 – 5; $MgCl_2$ – 1.2; succinic acid – 5). Readings for oxygen concentration in the medium after stabilization and "warming" of CM corresponded to the V2 respiration state – free respiration (sufficient amount of oxygen and substrate for oxidation in the medium, but the absence of the phosphorylation substrate – ADP). We measured the rate of oxygen consumption in the medium after adding 100 µl of 0.2 mM ADP solution into it (V3 respiration state) and after its consumption (V4 respiration state). Oxygen consumption by CM was calculated in nM O_2 / min / mg of protein in the sample. The

respiratory control (RC) ratio was calculated by dividing V3 respiration state to V4 [4].

The microviscosity of CM membranes was studied by assessing lateral diffusion in the hydrophobic region of the membranes using pyrene fluorescence and calculating its eximerization coefficients (C_E) $C_E=I_{470}/I_{390}$ at excitation wavelengths of 340 and 285 nm for lipid – lipid and protein – lipid interactions, respectively [7]. The intensity of pyrene dimer formation, characterized by C_E values, was inversely correlated with the membrane microviscosity. The peak luminescence was recorded at 390 nm for pyrene monomers and at 470 nm for pyrene dimers (excimers). The fluorescence of fluorophore was measured on the Cary Eclipse fluorescence spectrometer (Varian, USA).

Statistical processing of the obtained data was performed using the STATISTICA 10.0 software package. Normal distribution of quantitative data was checked using the Shapiro – Wilk test. Non-normal distribution of quantitative data was assessed by the non-parametric Mann – Whitney test. The results were presented as the median and the interquartile range *Me* (Q_1; Q_3). The differences were significant at $p < 0.05$.

RESULTS

Fig. 1 shows the results obtained in the assessment of CM respiration in the studied groups of animals. It can be seen that the groups of intact 2- and 15-month-old animals had no significant differences in the RC coefficient ($p = 0.05$). The value of this coefficient was 3.57 (3.32; 3.93) in 2-month-old rats and 3.36 (3.27; 3.40) in 15-month-old animals.

In the context of developed HF, the studied groups of animals differed significantly ($p < 0.01$) in the RC coefficient. At the same time, it was found that in 2-month-old animals, this coefficient remained virtually unchanged – 3.50 (3.19; 4.34) ($p = 0.86$). On the contrary, in 15-month-old animals with HF, the RC coefficient was significantly lower than in the intact animals of the same age and reached only 2.77 (2.71; 2.78) ($p < 0.05$) (Fig. 1).

When comparing C_E of pyrene fluorescence in areas of protein – lipid interactions in CM membranes of intact animals, no significant ($p = 0.11$) age-dependent differences were found (Fig. 2). On the contrary, when comparing the microviscosity coefficients of CM membranes in the zones of lipid – lipid interactions, an explicit age dependence was established (Fig. 3). In our study, the value of this coefficient in 2-month-old animals was 1.25 (1.01; 1.48), and in 15-month-old animals – 1.73 (1.37; 1.87) ($p < 0.001$).

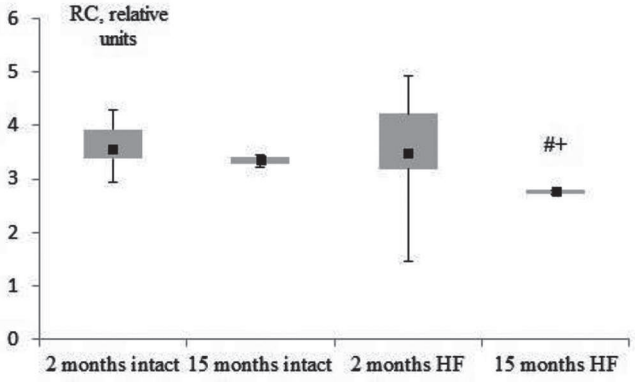

Fig. 1. Respiratory control coefficient (relative units) of rat cardiomyocytes; *Me* (Q_1; Q_3).

RC – respiratory control; HF – heart failure. Significant differences: # between age groups of animals with HF, $p < 0.01$; + between intact and experimental animals within one age group, $p < 0.05$

When comparing C_E of pyrene in the areas of protein – lipid interactions in myocardial cell membranes of animals with HF, a significant age-dependent decrease in this parameter was noted ($p < 0.05$). At the same time, in 2-month-old rats with HF, this coefficient, in comparison with intact animals, was significantly higher and reached 1.21 (1.06; 1.54) ($p < 0.01$). However, in 15-month-old animals with HF, this coefficient was found to be significantly lower and reached 0.88 (0.70; 0.90) ($p < 0.05$) (Fig. 2). In the zones of lipid – lipid interactions, no significant differences in the microviscosity coefficients between intact and experimental animals in both age groups were found.

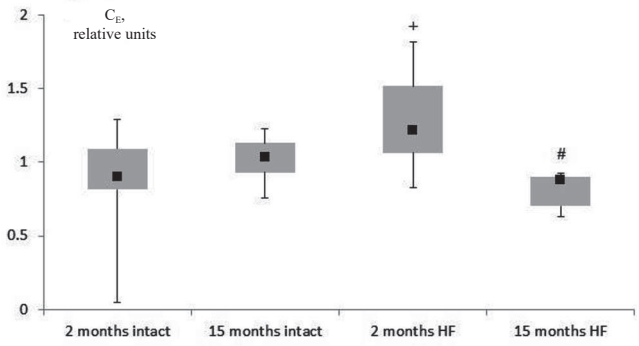

Fig. 2. Cardiomyocyte membrane eximerization coefficient (relative units) in zones of protein – lipid interactions, relative units; *Me* (Q_1; Q_3): C_E – eximerization coefficient; HF – heart failure (here and in Fig. 3); # significant differences between age groups of animals with HF, $p < 0.05$; + significant differences between intact and experimental animals within one age group, $p < 0.01$.

Besides, no significant difference was found in these coefficients between age groups when comparing both subgroups of intact rats and animals with modeled HF (Fig. 3).

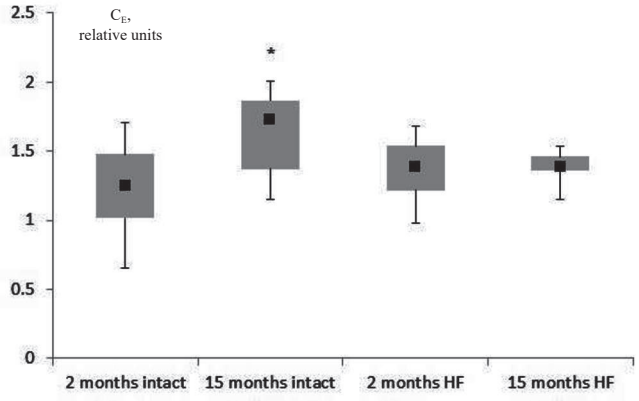

Fig. 3. Cardiomyocyte membrane eximerization coefficient (relative units) in zones of lipid – lipid interactions, relative units; *Me* (Q_1; Q_3): * significant differences between groups of intact 2- and 15-month-old animals, $p < 0.001$

DISCUSSION

The absence of significant differences in CM respiration between the intact animals of both age groups may be due to the activation of adaptive reserve capacities in CMs in adult animals. At the same time, according to the literature data, RC values in both groups fall within the reference norm (3–5 relative units) [8]. Interestingly, in HF in young animals, no significant change in RC was noted, which may indicate greater adaptive reserve capacities in CMs in young animals. In adult rats with HF, this parameter significantly decreased both in young animals with HF and in intact rats of the same age, which may be due to disruption of adaptive responses in cells and a decrease in function with age. It can also be assumed that the injection of toxic doses of isoproterenol increases synthesis of reactive oxygen species (ROS) in the cell in response to the effect of this agent due to intensification of energy production and an increase in electron leakage from the electron transport chain. It is in line with the results of our previous studies, which showed that the activity of antioxidant enzymes in the myocardium decreases with age [9].

The above hypothesis about the change in adaptive reserve capacities of myocardial cells is quite consistent with the data obtained from a comparative analysis of the CM membrane microviscosity in the same groups of animals. The differences in C_E between the groups of intact animals of different ages may be associated with age-dependent changes in cholesterol metabolism. One of the main functions of this neutral lipid is regulation of biological membrane viscosity by changing lateral movement of fatty acid residues in phospholipids [10]. With age, the level of cholesterol in the body increases [11], which may affect its content within the lipid bilayer of the membrane. The latter leads to an increase in the viscosity of membranes, which makes them rigid.

The data obtained in the groups of animals with HF could indicate oppositely directed age-dependent changes in the phospholipid composition of annular lipids, which is reflected in the C_E. These differences may affect the activity of cellular enzyme systems that provide energy metabolism.

CONCLUSION

Therefore, it was found that respiration in isolated CMs of intact animals is not subject to significant age-dependent changes. However, a trend toward its decrease has been noted. This may indicate depletion of compensatory mechanisms in the cell that maintain the optimal respiration level. With the development of HF, these differences become more pronounced, which manifests itself by a significant decrease in respiration in aged rats (15 months old) compared to intact animals of the same age group. At the same time, in 2-month-old rats with HF, the respiration level remains the same as in intact animals.

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