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Measurements of mitochondrial NADH pool and NADH production rate in acute brain slices and primary cell cultures using live cell imaging

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ABSTRACT

The paper presents results of the estimation of bioenergetic parameters in acute slices of brain rat by measuring mitochondrial pool and rate of NADH production using fluorescence imaging. NAD+ and NADH play crucial roles in mitochondrial energy metabolism due to their participation in the tricarboxylic acid cycle and in electron transport chain. That is why it is essential to know variability of its content in cells. In the study we used an approach based on the registration of auto-fluorescence of NADH. An experimental setup was designed and assembled for the imaging of the acute brain slices but also capable of measuring brain cell cultures. BDL-SMN-375 laser (Becker&Hickl, Germany) was used to excite fluorescence at the wavelength of 375 nm. The parameters of NADH content in both acute brain slices and cell cultures of midbrain and cortex were estimated. The obtained results can be of interest for better understanding of development of neurodegenerative diseases of mitochondrial disfunction origin.

Keywords: NADH, autofluorescence, neurodegenerative diseases, the mitochondrial pool of NADH, fluorescence imaging

1. INTRODUCTION

NADH and NAD+ are highly significant for cell vitality playing crucial role in the mitochondrial energy metabolism. Mitochondria take the lead in maintaining of homeostasis and producing of ATP in cells. That is why any pathological changes in mitochondrial function may leads to severe diseases and even death.[1,2] For instance, a great number of neurodegenerative disorders are connected with pathological processes in mitochondria.[3,4,5] Better understanding of the variability of the mitochondrial energy balance across brain regions can be of particular interest for the optimization the protection strategies against the action of mitochondrial toxins, pesticides and severe bioenergetic disbalance contributing to trigger dopaminergic neurodegeneration in Parkinson's disease.

NADH enables the respiratory chain to work as it is an electron carrier that donates electrons to complex I. Complex I oxidizes NADH on the inner side of the membrane in mitochondria while NAD+ reduction occurs in the matrix as a result of tricarboxylic acid cycle (TCA) and β-oxidation. [6] It should be noted out that NAD+ is the coenzyme for several enzymes in TCA. Besides, it may serve as a substrate for covalent protein modification while NADH takes part in the synthesis of a signalling molecule such as (cADPR) that mediate calcium homeostasis. Besides, they take a role in protection from oxidative stress and have antioxidation effect.[7,8,9]

Moreover, the transport of molecules through the mitochondrial membrane depends on NADH as it influences anion channels and is a component of MPT pores that form mitochondrial membrane potential.[10] In additional, NAD+ and NADH may also affect gene expression through several pathways and play a significant role in the cells aging.[11,12] It should be noted out that NAD+ also might be involved in apoptosis by selective inhibition of NAD synthesis.[13] To sum up, NADH and NAD+ are crucial for cells that is why their quantification may tell about the overall viability of cells.

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The brain has a very complex bioenergetic system which differs from other tissues. Brain cells are particularly sensitive to redox changes due to their high metabolic rate and limited antioxidant capacity.[14] Oxidative stress, inflammation, mitochondrial dysfunction, excitotoxicity, and impaired transcription have been identified as contributing factors in neurodegenerative disorders. Among these, mitochondrial dysfunction is central to the pathophysiology of chronic neurodegenerative disorders. For example, mitochondrial toxins such as rotenone and MPP + cause Parkinson's disease selectively damaging certain areas of the brain. Elucidation of the cellular specificity of brain regions and intercellular interactions in the bioenergetic system of the brain is also important for understanding the mechanisms underlying selective cellular vulnerability during neurodegeneration.[15] The study of in vivo cerebral metabolism at the microscopic level is important for understanding the function of the brain and its pathological changes.[16]

Fluorescence microscopy is one of the available methods for analyzing brain metabolism. Both oxidized NAD + and reduced NADH show strong UV absorption around 260 nm; however, only NADH absorbs noticeably in the 350–365 nm region and emits fluorescence with a peak at ~ 460 nm. Emitted NADH fluorescence as a useful endogenous, non-destructive marker of metabolic activity variation.[17,18] But the estimation of mitochondria metabolic activity cannot be done by the analysis only of NADH mitochondrial pool. It is important to determine at the same time the rate of NADH production in TCA and β-oxidation process.

Both brain sections and primary cell culture of neurons and astrocytes can be used as objects of research in the analysis of bioenergetics of brain cells. It depends on the aim of the research, used methods and equipment. This article demonstrates the promise of the fluorescence imaging technique for studying metabolic activity in brain tissues. The experimental setup developed for the study allowed us to reveal statistically significant differences in mitochondrial energy metabolism between the cortex and the midbrain in the measurements in acute brain slices. The data were also confirmed in studies with primary cell cultures of neurons and astrocytes from the same parts of the brain.

2. MATERIALS AND METHODS

Experimental part of the study was performed on brain acute slices of 12-week-old Wistar rats (n=5) and primary cell culture of neurons and astrocytes from midbrain and cortex regions of 0-3 days old Wistar rat pups (n=8). All animal work for isolation and preparation of brain slices was approved by the Institutional ethical committee of Orel State University ((Minutes No. 18 dated 21.02.2020) in compliance with Russian Federation legislation.

Euthanasia of 12-week-old Wistar rats was carried out by dislocation of the cervical spine. After extraction, the rat brain was placed in chilled HBSS. Horizontal sections of different brain regions (cortex and midbrain) with a thickness of 500 μm were cut, according to a standard procedures [19,20] and stored prior to experiments for at least 1 hour/37°C.

For imaging the acute slice was placed in an excavated slide with 150 μl of HEPES-buffered salt solution (HBSS). Acute slices were fixed mechanically to eliminate their movement during the experiment.

Primary cell cultures from the cortical and midbrain were prepared according to the standard protocol.[16] Cortex and midbrain were isolated and placed into ice-cold HBSS (Ca^{2+}, Mg^{2+}-free). The tissues were minced and trypsinised (0.1% for 15 min at 37°C), triturated and plated on poly-D-lysine-coated coverslips and cultured in Neurobasal A medium supplemented with B-27 and L-glutamine. Cultures were maintained at 37° C in a humidified atmosphere of 5% CO2 and 95% air, fed twice a week and maintained for a minimum of 12 days before experimental use to ensure the expression of glutamate and other receptors. All measurements on acute slice and cells were performed in 500 μl HBSS. FCCP (4 μM) and NaCN (5 mM) were used to determine the upper and lower levels of the mitochondrial NADH pool and rate of its production. The basic level of endogenous fluorescence, as well as its changes after addition of each reagent, were recorded. Each stage of measurement lasted for 3 minutes with an interval of 2 s between frames. The basic level was registered until the stabilization of the signal. After addition of uncoupler FCCP the respiration maximised. Consequently, oxidation of NADH into non-fluorescent NAD led to the lowest fluorescence signal. After that NaCN was added and the highest fluorescence signal was obtained since the substance specifically blocked the cytochrome oxidase (complex 4) and respiration was minimal.[21]

In the experimental imaging setup (Fig.1) the excitation radiation passed through optical fiber through collimator, bandpass excitation filter and reflected from the dichroic filter plate. In the fluorescent imaging channel, the fluorescence radiation from the sample passed through dichroic filter plate and was recorded by a high-sensitivity cooled CCD camera DCC 3260C (Thorlabs etc, USA). The camera field of view was about 1 mm^2 in focus plain on the surface of the sample.

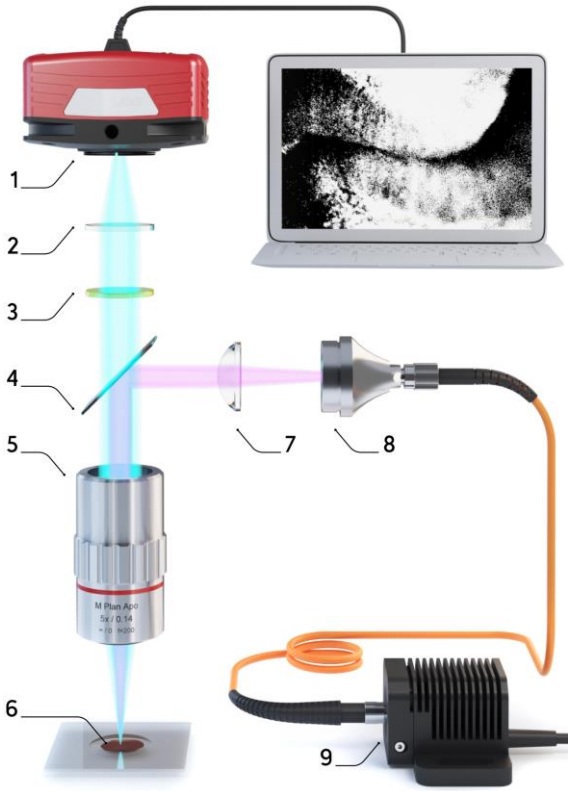

Figure 1. Layout of the setup for fluorescence imaging: 1 - CCD camera DCC 3260C; 2 - lens f=200 mm; 3 - fluorescence longpass light filter FGL400; 4 - dichroic filter MD416; 5 - Mitutoyo M Plan APO 5X planar apochromatic objective; 6 - sample; 7 - MF445-45 fluorescence filter; 8 - Thorlabs CVH100-COL collimator with LB1945-A lens; 9 – laser BDL-SMN-375.

3. RESULTS

The significance of statistical differences between the samples was assessed by Mann-Whitney U-test. Statistical analysis was performed using Origin Pro 2017 software. Discrepancies with $p < 0.05$ were considered to be significantly different. The NADH pool was calculated by subtracting of the lowest value of fluorescence (induced by FCCP) from the highest one observed after addition of the NaCN (Fig.2).

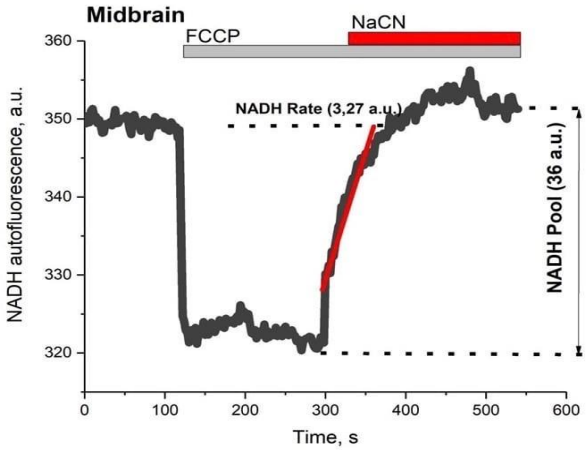

Figure 2. Representative trace of NADH autofluorescence from an acute slice from cortex.

The parameter is representative to characterize the activity of the electron transport chain and sensitive to the disruptions in mitochondrial functional state. A rate of NADH production was calculated by analysis of slopes of fluorescence after NaCN addition (Fig.2). The parameter depicts efficiency of the tricarboxylic acid cycle as NaCN blocks all respiration taking place in the cell. The obtained data are shown in the Fig. 3.

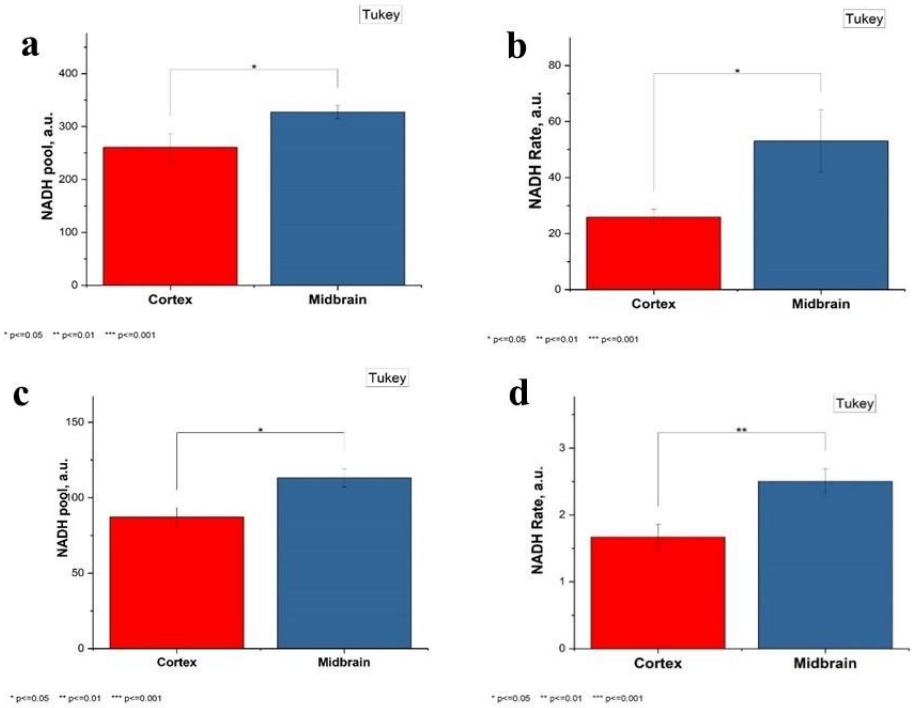

Figure 3. Difference in parameters of NADH pool (a) and rate of NADH production (b) assessed in cell cultures and acute slices (c) and (d).

The levels of the calculated parameters correlate for the measurements conducted in cell cultures and acute brain slices. Both NADH pool ($p<0.05$ for cell culture and brain slices) and the rate of NADH production ($p<0.05$ for cell culture and $p<0.01$ for brain slices) are found to be significantly higher in midbrain in comparison with the cortex (Fig.3). Recently it has been demonstrated that basal ATP level is similar in most of the brain regions (except cerebellum), whereas the ATP consumption is higher in midbrain neurons and astrocytes.[16] Thus, NADH pool in these cells, together with the rate of NADH production is expected to be elevated, which is apparently associated with similar or higher rate of mitochondrial respiration. More intensive metabolism and, consequently, higher consumption of NADH by cells in the midbrain in comparison to the cortex can explain the main observations of the study. Higher level of NADH consumption during mitochondrial respiration can be supported by the higher activity of NADH production in TCA as NADH rate data show. The results also explains higher vulnerability of midbrain to changes in the metabolism of mitochondria which leads to such neurodegenerative disease as Leigh syndrome.[22,23]

4. CONCLUSION

Mitochondria play a crucial role in processes that are central to cell physiology, bioenergetics, cell signaling, and cell death. In neurons, these functions are more vital. Therefore, the activity of neural cells is sensitive to the slightest changes in mitochondrial function. The analysed parameters of mitochondrial NADH pool and rate of NADH production are of particular interest for the quantitative characterization of the presence of the mitochondrial disfunction and overall status of the cell bioenergetics. Our measurements demonstrate that data obtained in primary cell culture highly correlate with one obtained in acute brain slices. According to our data, mitochondrial NADH pool and rate of NADH production are significantly higher in the cells of midbrain in comparison to the cortex. The results of the study can be used for

planning protection strategies for the treatment of neurodegenerative diseases taking into account the revealed interbrain variability in the parameters of abundance and production of a molecule playing major role in the cellular bioenergetics.

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REFERENCES

[1] Edeas, M. and Weissig, V., "Targeting mitochondria: Strategies, innovations and challenges. The future of medicine will come through mitochondria.," Mitochondrion 13(5), 389–390 (2013).
[2] López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. and Kroemer, G., "The hallmarks of aging," Cell 153(6), 1194–1199 (2013).
[3] Burchell, V. S., Gandhi, S., Deas, E., Wood, N. W., Abramov, A. Y. and Plun-Favreau, H., "Targeting mitochondrial dysfunction in neurodegenerative disease: Part II," Expert Opin. Ther. Targets 14(5), 497–511 (2010).
[4] Gandhi, S. and Abramov, A. Y., "Mechanism of oxidative stress in neurodegeneration," Oxid. Med. Cell. Longev. 2012 (2012).
[5] Lin, M. T. and Beal, M. F., "Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases," Nature 443(7113), 787–795 (2006).
[6] Ying, W., "NAD+/NADH and NADP+/NADPH in cellular functions and cell death: Regulation and biological consequences," Antioxidants Redox Signal. 10(2), 179–206 (2008).
[7] Ziegler, M., "New functions of a long-known molecule," Eur. J. Biochem. 267(6), 1550–1564 (2000).
[8] Mcguinnessf, E. T., Butlers, J. R. and Orange, S., "NAD+ KINASE," 1–11 (1985).
[9] Mazzio, E. A. and Soliman, K. F. A., "Cytoprotection of pyruvic acid and reduced β-nicotinamide adenine dinucleotide against hydrogen peroxide toxicity in neuroblastoma cells," Neurochem. Res. 28(5), 733–741 (2003).
[10] Bernardis, P., Vassanelli, S., Veronese, P., Colonna, R. and Szabo, I., "Modulation of the mitochondrial permeability transition pore. Effect of protons and divalent cations," J. Biol. Chem. 267(5), 2934–2939 (1992).
[11] Zhang, Q., Piston, D. W. and Goodman, R. H., "Regulation of corepressor function by nuclear NADH," Science (80-.). 295(5561), 1895–1897 (2002).
[12] Blander, G. and Guarente, L., "The Sir2 family of protein deacetylases," Annu. Rev. Biochem. 73, 417–435 (2004).
[13] Petit, P. X., Gendron, M. C., Schrantz, N., Métivier, D., Kroemer, G., Maciorowska, Z., Sureau, F. and Koester, S., "Oxidation of pyridine nucleotides during Fas- and ceramide-induced apoptosis in Jurkat cells: Correlation with changes in mitochondria, glutathione depletion, intracellular acidification and caspase 3 activation," Biochem. J. 353(2), 357–367 (2001).
[14] Bélanger, M., Allaman, I. and Magistretti, P. J., "Brain Energy Metabolism: Focus on Astrocyte-Neuron Metabolic Cooperation," Cell Metab. 14(6), 724–738 (2011).
[15] Qi, G., Mi, Y. and Yin, F., "Cellular Specificity and Inter-cellular Coordination in the Brain Bioenergetic System: Implications for Aging and Neurodegeneration ," Front. Physiol. 10, 1531 (2020).
[16] Cheng, X., Vinokurov, A. Y., Zherebtsov, E. A., Stelmashchuk, O. A., Angelova, P. R., Esteras, N. and Abramov, A. Y., "Variability of mitochondrial energy balance across brain regions," J. Neurochem. (2020).
[17] Gómez, C. A., Sutin, J., Wu, W., Fu, B., Uhlirova, H., Devor, A., Boas, D. A., Sakadžić, S. and Yaseen, M. A., "Phasor analysis of NADH FLIM identifies pharmacological disruptions to mitochondrial metabolic processes in the rodent cerebral cortex," PLoS One 13(3), e0194578 (2018).
[18] Heikal, A. A., "Intracellular coenzymes as natural biomarkers for metabolic activities and mitochondrial anomalies," Biomark. Med. 4(2), 241–263 (2010).
[19] Angelova, P. and Müller, W., "Oxidative modulation of the transient potassium current IA by intracellular arachidonic acid in rat CA1 pyramidal neurons," Eur. J. Neurosci. 23, 2375–2384 (2006).
[20] Novikova, I. N., Manole, A., Zherebtsov, E. A., Stavtsev, D. D., Vukolova, M. N., Dunaev, A. V., Angelova, P. R. and Abramov, A. Y., "Adrenaline induces calcium signal in astrocytes and vasoconstriction via activation of monoamine oxidase," Free Radic. Biol. Med. 159, 15–22 (2020).

[21] Bartolomé, F. and Abramov, A. Y., “Measurement of Mitochondrial NADH and FAD Autofluorescence in Live Cells,” Mitochondrial Med. 1, 263–270 (2015).
[22] Bonfante, E., Koenig, M. K., Adejumo, R. B., Perinjelil, V. and Riascos, R. F., “The neuroimaging of Leigh syndrome: case series and review of the literature,” Pediatr. Radiol. 46(4), 443–451 (2016).
[23] Wei, Y., Cui, L. and Peng, B., “Mitochondrial DNA mutations in late-onset Leigh syndrome,” J. Neurol. 265(10), 2388–2395 (2018).