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Functional State of the Mitochondria from Tissues of the Rat Brain after Chronic Occlusion of the Common Carotid Artery: Role of Lysyl Oxidase

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Stenosis and occlusion of the common carotid artery (CCA) are one of the main reasons of cerebrovascular pathologies; these factors determine the development of hypoperfusion of the brain. Disorders in the expression of lysyl oxidase (LOX) underlie the development of a number of pathological processes, including vascular and cerebral pathologies. Changes in the activity of this enzyme are assumed to significantly affect the functional state of the mitochondria (MCh). We examined the role of LOX in the regulation of energy metabolism in the rat brain under conditions of experimental unilateral chronic occlusion of the CCA (CCA ChO). Experiments were carried out on Wistar rats with ligated left CCA. Animals of one of the experimental groups received drinking water with 0.2% of a LOX blocker, β-aminopropionitrile (BAPN) during 8 weeks. After such course of BAPN introduction, we estimated the characteristics of energy metabolism in the MCh from tissues of the brain hemispheres using a polarographic technique. Occlusion of the CCA was accompanied by disorders in tissue respiration (oxidative phosphorylation in the MCh); these changes were more expressed in the left hemisphere ($P < 0.05$), but those in the right one were also quite noticeable. There were indications for the existence of interhemisphere differences in the functioning of the MCh complex 1 in healthy control animals; the respective values were greater in the left hemisphere. Introduction of BAPN promoted partial recovery of the MCh functions; this was manifested in some weakening of the effects of CCA occlusion. Thus, under conditions of unilateral CCA ChO, energy metabolism in both brain hemispheres undergoes negative changes. Changes in the LOX activity are one of the factors responsible for negative shifts in the indices of MCh functioning related to hypoperfusion of brain tissues.

Keywords: lysyl oxidase (LOX), BAPN (a LOX blocker), brain, hypoperfusion, mitochondria (MCh), energy metabolism, polarography.

INTRODUCTION

As is generally known, pathological changes in the precerebral vessels increase the risks of stroke. Stenoses and occlusions of the brachio-cephalic arteries result in the development of more or less intense hypoperfusion of the brain, and this can ultimately result in insult [1]. Chronic hypoperfusion of the brain, naturally, is characterized by slowing down of cerebral circulation and decreases in the levels of oxygen and glucose in the cerebral blood. These modifications, together with other pathophysiological changes, result in impairments and death of the cerebral neurons [2, 3].

The functional state of the mitochondrial (MCh) apparatus in cerebral tissues and neuronal activity in the CNS are closely interrelated [4]. More than 90% of the requirements of nerve cells for energy resources (ATP) are provided by oxidative phosphorylation in the MCh using glucose as a substrate and oxygen as an oxidant. A sufficient amount of energy generated by the MCh is of crucial importance for the maintenance of main cell functions in the norm [5, 6]. Disorders in the cell supply by glucose and oxygen result in significant disorders of the MCh functions [7]; such disorders play the role of one of the main pathophysiological factors in acute and chronic diseases of the CNS [8].

An enzyme, lysyl oxidase (LOX), catalyses cross-linking of collagen molecules at the expense of oxidation of primary amines. This process is crucially important for the stabilization of collagen

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fibrillae and also of fibers of mature elastin; this is responsible for the maintenance of functional characteristics of connective tissue, a few processes of embryonal development, and remodeling of tissues of an adult organism [9]. It is important to note that such active compounds as hydrogen peroxide and ammonia are produced in noticeable amounts as side products of the above-mentioned catalytic reactions. In addition, LOX regulates other intracellular functions; in particular, it is involved in the control of cell differentiation, mobility/migration of the cells, and transcription of some genes. The catalytic activity of LOX can be specifically and irreversibly inhibited by β-aminopropionitrile (BAPN) [10]. It is known that expression of LOX is controlled by hypoxic-inducible factors (HIFs) [11]. As was found, LOX functions as a regulator of hypoxia-induced progressive development of tumors. This effect is realized via an HIF-1-dependent mechanism and manifested in the case of breast cancer, prostate cancer, renal cell carcinoma, and a few tumors of the head, neck, and brain [12, 13]. Simultaneously, a significant role of LOX was demonstrated in the development of cardiovascular diseases, in particular atherosclerotic modifications of the vessels, and formation of intracranial aneurysms [14, 15]. There are certain data that LOX activity increases under conditions of oxidative stress [16]. Experiments carried out *in vitro* and *in vivo* showed that the activity of this enzyme influences the processes of angiogenesis; the expression of vascular endothelial growth factor (VEGF) is affected in this case [17]. It should, nonetheless, be recognized that the level of involvement of LOX in a few processes of cerebrovascular pathology remains at present poorly elucidated. The statement that changes in the LOX activity are capable of inducing considerable disorders of normal functioning of the MCh under conditions of insufficient perfusion of the brain appears to be rather well based. In particular, this can be realized under conditions of chronic occlusion (ChO) of the common carotid artery (CCA).

We tried to elucidate the effects induced by a change in the LOX activity on the indices of energy metabolism in the MCh obtained from tissues of the brain hemispheres; in experimental rats, unilateral CCA ChO was performed.

METHODS

Experiments were carried out on Wistar rats (body mass 250 to 300 g). Animals were kept in the vivarium of the Bogomolets Institute of Physiology of the NAS of Ukraine; standard food/water supply and illumination regimen (12/12 h) were provided.

Three experimental groups were formed, group 1 (control intact rats, $n = 6$), group 2 (rats with CCA ChO, $n = 6$), and group 3 (rats with CCA ChO treated with BAPN, $n = 6$). In the latter group, BAPN, a selective LOX blocker, was introduced perorally (0.2% in drinking water). An operation providing CCA ChO and performed under aseptic conditions corresponded to ligation of the left CCA at its middle level with subsequent suturing of the wound; ketamine anesthesia (1 ml per 300 g) was used.

Eight weeks after CCA ChO, the energy metabolism in the MCh obtained from tissues of the left and right brain hemispheres was examined. After decapitation of the animal, the brain was washed out with cold (4°C) 0.9% KCl solution, dispersed, and homogenized in a separation medium of the following composition (mM): saccharose, 250; Tris HCl, 10, and EDTA, 1.0 with the addition of 0.1% BSA (pH 7.4) [18]. Mitochondria were separated from the tissue using a standard technique of differential centrifugation in the separation medium.

Processes of respiration and oxidative phosphorylation in the MCh obtained from each of the two hemispheres were examined using a polarographic technique (by Chance); a closed polarographic oxygen sensor (Clark electrode), and an Oxygraph device were used. The measurements were performed at 26°C. The incubation medium contained (mM): saccharose, 300; $Na_2H_2PO_4$, 5.0, and Tris HCl, 2.0 (pH 7.4). Sodium succinate (5.0 mM), sodium glutamate (5.0 mM), and sodium malate (2.5 mM) were used as oxidation substrates. Rotenone (2.0 mM) was used as an inhibitor of the MCh enzyme complex I. The process of respiration was stimulated by the addition of 200 μM ADP in the polarographic cell. The amount of protein was estimated by the Lowry technique [20].

According to the obtained chrono-amperographic curves, the parameters of respiration of the MCh were calculated. These were: (i) rate of oxygen consumption in the resting state and in the absence of ADP (V2); (ii) rate of respiration with phosphorylation (in the metabolic state 3, by

Chance, V3); (iii) rate of controlled respiration of MCh in the metabolic state 4 (V4); (iv) coefficient of respiratory control, by Chance (V3/V4), and (v) coefficient of efficacy of phosphorylation (ADP/O) [21]. Reagents provided by Sigma (USA) were used.

Qualitative data were treated statistically using the Student's *t*-test; in estimation of intergroup differences, $P < 0.05$ was considered the index of significance.

RESULTS

Indices of MCh Functioning after CCA ChO. Analysis of the functional properties of the MCh obtained from brain tissues of rats with CCA ChO demonstrated the following. Under above conditions, the MCh from both hemispheres showed a lower intensity of ADP-stimulated respiration (V3) in the case where succinate + rotenone were used as the substrate for oxidation. In the MCh from the left and right hemispheres, the decrements were 28.4 and 15.0% ($P < 0.05$), respectively, as compared with the analogous indices in the control. Indices of respiratory control (V3/V4) in the above-mentioned hemispheres were smaller by 31.5 and 21.0%, respectively ($P < 0.05$), and this was observed against the background of drops in the efficiency of phosphorylation (ADP/O; in the left and right hemispheres, this index was lower by 10 and 6%, respectively; $P < 0.05$) (Fig. 1).

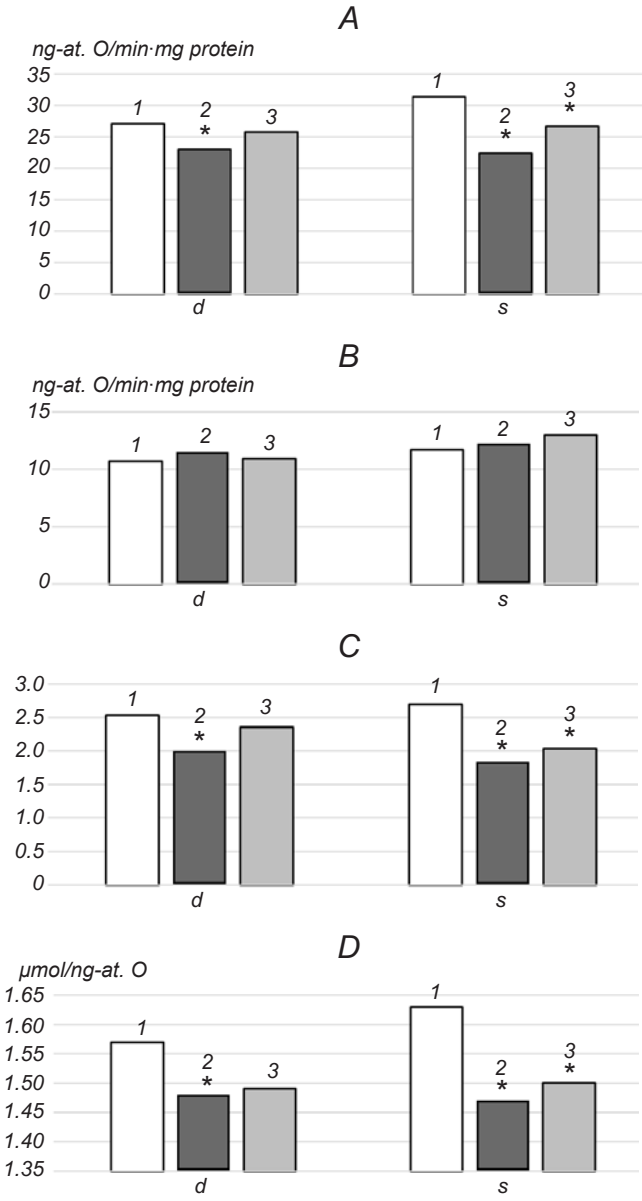
When glutamate+malate were used as the substrate for oxidation, analogous changes in the indices of energy metabolism were observed in the MCh from both hemispheres. The intensity of ADP-stimulated respiration (V3) in these subcellular structures was lower (by 34.5% in the MCh from the left hemisphere and by 23.5%, on average, in those from the right one; $P < 0.05$). Indices of respiratory control (V3/V4) in MCh from these hemispheres were lower by 50 and 46%, respectively ($P < 0.05$). Simultaneously, the efficacy of using O_2 (ADP/O) in tissues of both hemispheres after left-side occlusion of the CCA was noticeably lower, as compared with the control (by 12.7 and 10.0%, respectively) (Fig. 2).

Changes in the respiration indices observed in MCh when using oxidation substrates for complexes I and II in the respiratory chains of these organelles were more intense on the side of CCA occlusion (in the left hemisphere); for complex I, these changes were more considerable (Figs. 1 and 2).

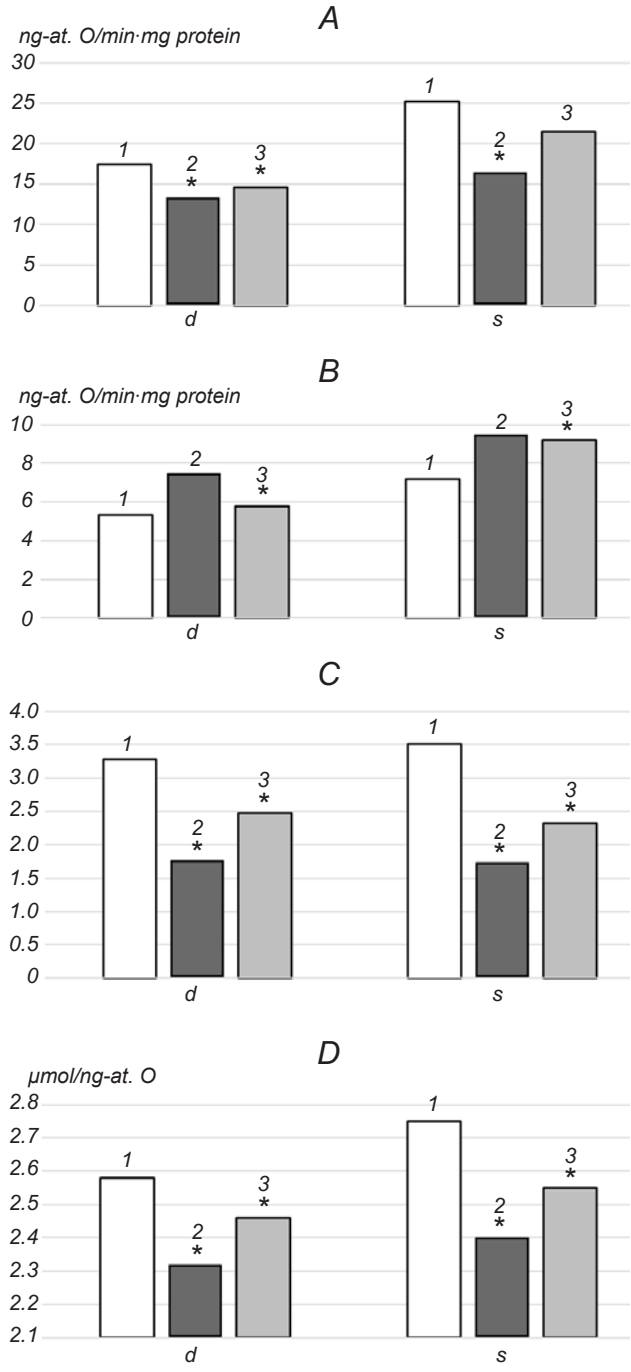
It should be mentioned that a noticeable interhemisphere asymmetry of the MCh respiratory function was observed under control conditions in the case where glutamate+malate were used. The mean V3 value in MCh from the left hemisphere was higher than that from the opposite side by 32.0%, while for the V4 the respective difference was 26.4% ($P < 0.05$).The efficacy of phosphorylation in the "left" MCh differed from the respective value for the right ones by 6.2% ($P < 0.05$) (Fig. 2).

Effects of BAPN on the Functional Indices of MCh after CCA ChO. The LOX blocker BAPN (0.02% aqueous solution) was introduced into the organism of experimental animals during drinking within a 8-week-long period. Analysis of the functional properties of MCh in the corresponding group showed that, when using succinate+rotenon as the oxidation substrate, the intensity of ADP-stimulated respiration (V3) was lower in MCh from both hemispheres (left, by 15.1%; right, by 5.2%; $P < 0.05$ in both cases). Indices characterizing respiratory control (V3/V4) for the left and right hemispheres were also lower (by 24.2 and 6.8%, respectively; $P < 0.05$). This was observed against the background of drops in the efficacy of phosphorylation (ADP/O). In the left hemisphere, the decrement was 8.0%, while in the right one, this was 5.1% ($P < 0.05$) (Fig. 1). Comparison with the data obtained under conditions of isolated action of CCA ChO showed that the indices characterizing MCh functioning increased somewhat. In the left hemisphere, increments for the above-mentioned indices were 13.3, 7.3, and 2.0%, respectively. In the right hemisphere, the corresponding values were 9.8, 14.2% ($P < 0.05$), and 0.9%.

If glutamate+malate were used as the substrate for oxidation, similar changes were observed in both brain hemispheres. The decrement in the intensity of ADP-stimulated restoration (V3) was equal to 14.7 and 16.1% ($P< 0.05$) in the left and right hemispheres, respectively. Considerable decreases in the index of the respiratory control (V3/V4) were 33.7 and 24.4% in MCh from these hemispheres ($P < 0.05$ in both cases). The efficacy of using O_2 (ADP/O) was also lower, by 7.3% at the left and 4.7% at the right, on average ($P < 0.05$). In this case, BAPN recovered, to a noticeable extent, the indices of MCh functioning. The respective differences were 19.8, 16.3, and 5.4%, and 7.4, 21.6, and 5.3%, on average, as compared with the analogous indices in the CCA ChO group, respectively ($P < 0.05$) (Fig. 2).



F i g. 1. Mean values of the indices of respiration of the mitochondria from tissues of brain hemispheres (*d*, right and *s*, left) of rats after occlusion of the common carotid artery (ACC); succinate+rotenone were used as the substrate for oxidation. A and B) Metabolic states 3 and 4 (V3 and V4, respectively), by Chance; C) index of respiratory control (V3/V4); D) efficacy of using O_2 (ADP/O). 1) Control, 2) after occlusion of the left ACC, and 3) after ACC occlusion and a course of peroral introduction of a lysyl oxidase blocker, BAPN. Asterisks show cases of significant differences from the control values ($P< 0.05$).



F i g. 2. Mean values of the indices of respiration of the mitochondria from tissues of brain hemispheres; glutamate+ +malate were used. Designations are similar to those in Fig. 1.

DISCUSSION

As is generally known, MCh are one of the main targets subjected to impairment by free radicals under hypoxia/ischemia conditions; this inevitably leads to significant decreases in the production of energy by the MCh respiratory chain [22]. Our data agree with the results of studies of the MCh complex I functioning in the brain of primates [23]. The respective experiments showed that the activity of this complex decreases under conditions of ischemia/reperfusion of the brain; this effect is related to suppression of the respiration processes in the MCh. Clear disorders of the MCh functions in strokes are manifested as a drop in the intensity of oxidative phosphorylation, intensified production of reactive oxygen species (ROSs), and disorders of calcium homeostasis in the MCh. This ultimately can lead to increased neuronal death [24–26]. Under conditions of chronic hypoperfusion of the brain, transport of oxygen to the tissues decreases, the intensity of oxidative phosphorylation drops, and the activity of complexes I and II in MCh is suppressed [27]. Considerable decreases in the rate of consumption of oxygen in the presence of oxidation substrates for complexes I and II under conditions of brain ischemia are indicative of significant disorders of the processes of oxidative phosphorylation in the MCh; the respective effects were mentioned in our earlier publication [28].

Moreover, MCh dysfunction may result from the impairment of some mechanisms providing the functioning of the blood-brain barrier. In particular, such impairments can be related to changes in the function of a specific protein, ATP-binding cassette transporter (ABC-transporter). Such shifts can be crucial factors for the development of a few pathological states [29]. In particular, the ABCB10-transporter has been identified as a part of the probable mechanism capable of preventing the development of oxidative stress [30]. Decreased activity of the ABCB10-transporter was observed in some neurodegenerative diseases [31, 32]. As is believed, disorders in the activity of the ABC-transporter result in weakening of the process of detoxication in certain cerebral regions. Such a situation is believed to be related to the impairment and death of neurons in the *substantia nigra* in Parkinson's disease, cells of the caudate nucleus in Huntington's disease, and neurons of the hippocampus and temporal cortex in Alzheimer's disease [33].

Under stroke conditions, a number of disorders develops in the intracellular redox status and calcium homeostasis in the MCh [34]. An excess of Ca^{2+} ions results in progressive MCh dysfunction; the latter is mediated by disorders in the activity of calcium-dependent enzymes (e.g., of mtNOS). As was shown, mtNOS is functionally connected with complex I of the MCh respiratory chain. Upon activation of complex I, mtNOS is also active and generates noticeable amounts of NO using reverse electron transfer [35]. The inactivation of complex I results in considerable disorders of the functioning of mtNOS. This enzyme begins to produce ROSs (i.e., it begins to function as a pro-oxidant agent) and is involved, in such a way, in the development and enhancement of oxidative stress [36]. In the case of strong overloading of the MCh by Ca^{2+} ions, the intensity of processes in the MCh respiratory chain within a segment of complex I decreases. Under conditions of blocking of this complex, tissue respiration is realized via a "backway," namely via complex II of the MCh respiratory chain [37].

Disorders in the functioning of the MCh under conditions of brain hypoperfusion on the side contralateral to that of CCA ligation may result from the development of the so-called ischemic steal syndrome. Some peculiarities of the development of the great arterial circle (which, in general, can be a variant of the norm) may provide partial supply of circulation in the hemispheres at the expense of contralateral vessels. The functioning of the latter upon stenosis/occlusion of the CCA is impaired. Under conditions of the normal development of the great arterial circle, the blood volume coming to the brain is redistributed between regions with intact blood supply and regions with decreased blood supply. This, finally, results in decreases in the volumes of circulating blood in both ipsi- and contralateral hemispheres and, consequently, in certain deviations of the MCh functioning in both hemispheres.

The interhemisphere dissimilarities of the activity of the MCh complexes, which were noticed in our experiments, may, probably, be related to different general intensities of the functioning of brain hemispheres and specificities of the functions performed by these hemispheres.

Lysyl oxidase is an enzyme catalyzing the formation of mutual connections of molecules of extracellular matrix proteins (collagen and elastin). This enzyme is characterized by certain specificities of the extra- and intracellular distribution in the

cerebral structures [38]. Being an extracellular protein, LOX is expressed in the brain of rats and mice in vascular plexuses, vascular walls, and glial matrix of the brain. In neurons, LOX is localized in the cytoplasm. It was found that LOX activity in the hippocampus and walls of the cerebral vessels is noticeably intensified in Alzheimer's disease and dementias [388, 39].

In the cases of CNS impairments of one nature or another, LOX is actively synthesized in and excreted by the cells in close proximity to the impaired regions. This enzyme is also involved in the modulation of functions of the extracellular matrix; this can be accompanied under pathological conditions by considerable accumulation of mature collagen fibers and the formation of scars. As was shown, enzymatic activity of LOX is also highly increased in the regions of brain tissue damage [40]. Changes in the extracellular matrix and formation of scars after CNS impairments can appear excessively intense, and this prevents regeneration of the axons and functional recovery of the nerve cells. In turn, inhibition of LOX by BAPN promoted the process of recovery, but manifestations of regeneration of the axons were absent [41].

The above-mentioned enzyme was also identified as a potential significant source of ROS production; in oxidative stress, an increase in its activity was observed [42]. As was demonstrated, activation of HIF-1α occurs under conditions of brain ischemia; this factor, in turn, promotes intensification of LOX expression and increase in the activity of this enzyme [43].

Blocking of LOX by BAPN under conditions of CCA ChO in our experiments provided some positive changes in the MCh functioning; the respective indices were partly recovered. This may develop due to a certain decrease in the intensity of ROS production. The increase in the amount of hydrogen peroxide produced as a side product in the course of LOX functioning, probably, also noticeably affects the state and functions of the MCh and is responsible for certain disorders in their functioning. Results of studies of superoxide dismutase (mSOD1) in transgenic mice G93A were indicative of increases in the LOX activity and amount of *LOX*-mRNC in the brain and spinal cord of these animals [23]. These facts show that changes in the LOX activity are interconnected with the pathological process and that such changes are mediated (at least partly) by intense ROS production. Another strain of transgenic mice with intensified expression of LOX in vascular smooth muscle cells (TgLOX) allows one to assume that there are two stable practically indistinguishable phenotypes. As was shown, TgLOX mice are characterized by abnormally high expression of LOX in the carotid arteries and aorta, and this is accompanied by remodeling of the vessels [44]. The intensified expression of LOX in these mice correlated with increases in the amounts of oxidative stress markers (increased levels of H_2O_2 and O_2), increase in the NADPH oxidase level, and MCh dysfunction. At the same time, blocking of the LOX activity by BAPN prevented the development of the mentioned effects [42].

Therefore, it can be concluded that unilateral CCA ChO promotes the development of disorders in the processes of tissue respiration and production of energy by the MCh in both brain hemispheres, while such changes are more intense in the hemisphere supplied by the occluded vessel. Prevention of disorders in MCh functioning in cerebral tissues against the background of CCA ChO provided by treatment with BAPN (a LOX blocker) is indicative of the fact that LOX activity is directly involved in the development of the above functional disorders under the respective pathogenetic conditions.

REFERENCES

1. W. J. Powers, G. A. Press, R. L. Grubb Jr., et al., "The effect of hemodynamically significant carotid artery disease on the hemodynamic status of the cerebral circulation," *Ann. Int. Med.,* **106**, 27–34 (1987).
2. A. A. Boldyrev, "Role of active oxygen species in the vital activity of a neuron," *Usp. Fiziol. Nauk,* **34**, No. 3, 21–34 (2003).
3. Ye. I. Gusev and V. I. Skvortsov, *Ischemia of the Brain*, Meditsina, Moscow (2001).
4. A. P. Kudin, T. A. Kudina, J. Seyfried, et al., "Seizure-dependent modulation of mitochondrial oxidative phosphorylation in rat hippocampus," *Eur. J. Neurosci.,* **15**, No. 7, 1105–1114 (2002).
5. T. Ohtsuki, M. Matsumoto, K. Suzuki, et al., "Mitochondrial lipid peroxidation and superoxide dismutase in rat hypertensive target organs," *Am. J. Physiol.,* **268**, H1418–H1421 (1995).
6. W. S. Kunz, I. V. Goussakov, H. Beck, et al., "Altered mitochondrial oxidative phosphorylation in hippocampal slices of kainate-treated rats," *Brain Res.,* **826**, No. 2, 236–242 (1999).
7. D. Yates, "Cell biology of the neuron: fuelling transport," *Nat. Rev. Neurosci.,* **14**, No. 3, 156 (2013).
8. O. Kann, "The interneuron energy hypothesis: Implications for brain disease," *Neurobiol. Dis.,* **90**, 75–85 (2016).

9. K. Csiszar, "Lysyl oxidases: a novel multifunctional amine oxidase family," *Prog. Nucleic Acid Res. Mol. Biol.,* **70**, 1–32 (2001).
10. L. I. Smith-Mungo and H. M. Kagan, "Lysyl oxidase: properties, regulation and multiple functions in biology," *Matrix Biol.,* **16**, No. 7, 387–398 (1998).
11. J. T. Erler, K. L. Bennewith, M. Nicolau, et al., "Lysyl oxidase is essential for hypoxia-induced metastasis," *Nature*, **440**, 1222–1226 (2006).
12. R. Schietke, C. Warnecke, I. Wacker, et al., "The lysyl oxidases LOX and LOXL2 are necessary and sufficient to repress E-cadherin in hypoxia: insights into cellular transformation processes mediated by HIF-1," *J. Biol. Chem.,* **285**, No. 9, 6658–6669 (2010).
13. R. da Silva, M. Uno, S. K. Marie, and S. M. Oba-Shinjo, "LOX expression and functional analysis in astrocytomas and impact of IDH1 mutation," *PLoS One,* **10**, No. 3, e0119781 (2015).
14. C. Rodriguez, J. Martmez-Gonzalez, B. Raposo, et al., "Regulation of lysyl oxidase in vascular cells: lysyl oxidase as a new player in cardiovascular diseases," *Cardiovasc. Res.,* **79**, No. 1, 7–13 (2008).
15. H. Akagawa, A. Narita, H. Yamada, et al., "Systematic screening of lysyl oxidase-like (LOXL) family genes demonstrates that LOXL2 is a susceptibility gene to intracranial aneurysms," *Human Genet.,* **121**, Nos. 3/4, 377–387 (2007).
16. M. Majora, T. Wittkampf, B. Schuermann, et al., "Functional consequences of mitochondrial DNA deletions in human skin fibroblasts: increased contractile strength in collagen lattices is due to oxidative stress-induced lysyl oxidase activity," *Am. J. Pathol.,* **175**, No. 3, 1019–1029 (2009).
17. A. M. Baker, D. Bird, J. C. Welti, et al., "Lysyl oxidase plays a critical role in endothelial cell stimulation to drive tumor angiogenesis," *Cancer Res.,* **73**, No. 2, 583–594 (2013).
18. A. L. Ton'shin, N. V. Lobysheva, L. C. Yaguzhinskii, et al., "Effect of the inhibitory neuromediator glycine on slow destructive processes in slices of the cerebral cortex at anoxia," *Biokhimiya,* **72**, No. 5, 631–641 (2007).
19. B. Chance and G. Williams, "The respiratory chain and oxidative phosphorylation," *Adv. Enzymol.,* **17**, 65–134 (1956).
20. R. W. Estabrook, "Mitochondrial respiratory control and the polarographic measurement of ADP:O ratio," *Methods Enzymol.,* **10**, 41–47 (1967).
21. *Manual for Studies of Biological Oxidation using a Polarographic Technique*, V. G. Frank, ed., Nauka, Moscow (1971).
22. L. D. Lyk'yanova, "Bioenergetic hypoxia: Meaning, mechanisms, and ways of correction," *Byul. Eksp. Biol. Med.,* **124**, No. 9, 244–254 (1997).
23. H. Tsukada, "The use of ^{18}F-BCPP-EF as a PET probe for complex I activity in the brain," *Methods Enzymol.,* **547**, 417–431 (2014).
24. E. T. Chouchani, V. R. Pell, E. Gaude, et al., "Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS," *Nature,* **515**, No. 7527, 431–435 (2014).
25. D. N. Doll, H. Hu, J. Sun, et al., "Mitochondrial crisis in cerebrovascular endothelial cells opens the blood-brain barrier," *Stroke,* **46**, No. 6, 1681–1689 (2015).
26. A. C. Schinzel, O. Takeuchi, Z. Huang, et al., "Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia," *Proc. Natl. Acad. Sci. USA,* **102**, No. 34, 12005–12010 (2005).
27. J. C. de la Torre, "Are major dementias triggered by poor blood flow to the brain? Theoretical considerations," *J. Alzheimers Dis.,* **57**, No. 2, 353–371 (2017).
28. K. R. Dave, I. Saul, R. Busto, et al., "Ischemic preconditioning preserves mitochondrial function after global cerebral ischemia in rat hippocampus," *J. Cerebr. Blood Flow Metab.,* **21**, No. 12, 1401–1410 (2001).
29. J. Pahnke, C. Fröhlich, M. Krohn, et al., "Impaired mitochondrial energy production and ABC transporter function – A crucial interconnection in dementing proteopathies of the brain," *Mech. Ageing Dev.,* **134**, No. 10, 506–515 (2013).
30. M. Liesa and W. Qiu, "Shirihai mitochondrial ABC transporters function: the role of ABCB10 (ABC-me) as a novel player in cellular handling of reactive oxygen species," *Biochim. Biophys. Acta,* 1823, No. 10, 1945-1957 (2012).
31. M. Krohn, C. Lange, J. Hofrichter, et al., "Cerebral amyloid-P proteostasis is regulated by the membrane transport protein ABCC1 in mice," *J. Clin. Invest.,* **121**, No. 10, 3924–3931 (2011).
32. D. M. van Assema, M. Lubberink, P. Rizzu, et al., "Blood- brain barrier P-glycoprotein function in healthy subjects and Alzheimer's disease patients: effect of polymorphisms in the ABCB1 gene," *EJNMMI Res.,* **2**, No. 1, 57 (2012).
33. A. L. Bartels, A. T. Willemsen, R. Kortekaas, et al., "Decreased blood-brain barrier P-glycoprotein function in the progression of Parkinson's disease, PSP and MSA," *J. Neural Transm.,* **115**, No. 7, 1001–1009 (2008).
34. Y. Liao, Y. Dong, and J. Cheng, "The function of the mitochondrial calcium uniporter in neurodegenerative disorders," *Int. J. Mol. Sci.,* **18**, No. 2, 248–252 (2017).
35. S. S. Bombicino, D. E. Iglesias, T. Zaobornyj, et al., "Mitochondrial nitric oxide production supported by reverse electron transfer," *Arch. Biochem. Biophys.,* **607**, 8–19 (2016).
36. M. S. Parihar, A. Parihar, F. A. Villamena, et al., "Inactivation of mitochondrial respiratory chain complex I leads mitochondrial nitric oxide synthase to become pro-oxidative," *Biochem. Biophys. Res. Commun.,* **367**, No. 4, 761–767 (2008).
37. M. S. Parihar, R. R. Nazarewicz, E. Kincaid, et al., "Association of mitochondrial nitric oxide synthase activity with respiratory chain complex I," *Biochem. Biophys. Res. Commun.,* **366**, No. 1, 23–28 (2008).
38. G. M. Gilad, H. M. Kagan, and V. H. Gilad, "Evidence for increased lysyl oxidase, the extracellular matrix-forming enzyme, in Alzheimer's disease brain," *Neurosci. Lett.,* **376**, No. 3, 210–214 (2005).

39. P. A. Li, Q. He, T. Cao, et al., "Up-regulation and altered distribution of lysyl oxidase in the central nervous system of mutant SOD1 transgenic mouse model of amyotrophic lateral sclerosis," *Mol. Brain Res.*, **120**, No. 2, 115–122 (2004).
40. G. M. Gilad, H. M. Kagan, and V. H. Gilad, "Lysyl oxidase, the extracellular matrix-forming enzyme, in rat brain injury sites," *Neurosci. Lett.,* **310**, No. 1, 45–48 (2001).
41. G. M. Gilad and V. H. Gilad, "Beta-aminopropionitrile treatment can accelerate recovery of mice after spinal cord injury," *Eur J. Pharmacol.,* 430, No. 1, 69-72 (2001).
42. S. Martinez-Revelles, A. B. Garcia-Redondo, M. S. Avendano, et al., "Lysyl oxidase induces vascular oxidative stress and contributes to arterial stiffness and abnormal elastin structure in hypertension: role of p38MAPK," *Antioxid Redox Signal,* **27**, No. 7, 379–397 (2017).
43. A. Guadall, M. Orriols, J. F. Alcudia, et al., "Hypoxia-induced ROS signaling is required for LOX up-regulation in endothelial cells," *Front. Biosci. (Elite Ed.),* **1**, No. 3, 955–967 (2011).
44. M. Orriols, A. Guadall, M. Galân, et al., "Lysyl oxidase (LOX) in vascular remodelling. Insight from a new animal model," *Thromb. Haemost.,* **112**, No. 4, 812–824 (2014).