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Adaptation to Hypoxia Prevents Disturbances in Cerebral Blood Flow during Neurodegenerative Process

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The rats with neurodegenerative brain disorder induced by administration of a toxic fragment of β-amyloid demonstrate weakened endothelium-dependent dilation of cerebral vessels, which attested to impaired production of endothelial NO. At the same time, toxic β-amyloid fragment induced the formation of NO depots in the walls of cerebral vessels, which indirectly attests to NO overproduction in the brain tissue. Preadaptation to hypoxia prevented endothelial dysfunction and improved the efficiency of NO storage. Our results suggest that adaptation to hypoxia protects the brain from various changes in NO production during neurodegenerative damage.

Key Words: *nitric oxide; neurodegenerative brain disorders; nitric oxide storage; dinitrosyl iron complexes*

NO stress induced by massive NO overproduction in the microglia and astrocytes largely contributes to the development of neurodegenerative processes in the brain [5]. On the other hand, NO deficiency in the endothelium of cerebral vessels leads to impairment of blood supply to brain cells and increases the severity of neurodegenerative processes [12]. Alzheimer's disease (AD) is a vascular injury with neurodegenerative complications [12]. NO can serve as a neurotoxic or neuroprotective factor in various neurodegenerative diseases, including AD and Parkinson's disease. Therefore, it is important to develop new methods for stimulation or inhibition of NO production.

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The most effective approach to NO modulation is adaptation to hypoxia stimulating NO synthesis and preventing not only NO deficiency and endothelial dysfunction, but also NO overproduction [9]. Adaptive protection from NO overproduction is based on two mechanisms: prevention of NO overproduction is related to prestimulation of NO synthesis and accumulation by the negative feedback mechanism; various types of adaptation are accompanied by the formation of NO stores in the vascular wall, presented by NO-containing complexes, mainly dinitrosyl iron complexes (DNIC) and nitrosothiols [2]. NO stores play an important role in adaptation of the cardiovascular system, because they serve as an additional nonenzymatic source of NO compensating for NO deficiency and binding excess NO under conditions of NO overproduction [2,9].

The role of free and stored NO in adaptation of the peripheral vascular system to hypoxia was

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extensively studied [9]. The role of NO in adaptation to brain hypoxia is less understood, but there are published data on the existence of NO-dependent mechanisms for brain adaptation to hypoxia. It was shown that adaptation to hypoxia stimulates NO synthesis in the brain [8], while NO synthesis inhibitors prevent adaptation under conditions of long-term intermittent hypoxia or hypoxic preconditioning of various disorders, including severe cerebral ischemia [14], experimental epileptogenesis [4], sublethal hypobaric hypoxia [8], and cognitive dysfunction in rats with experimental AD [1].

Here we studied NO-dependent changes in cerebral blood flow during experimental neurodegenerative disorder of the brain (AD). We also evaluated the possibility of preventing these disturbances using adaptation to hypoxia and analyzed the role NO depots in the formation of adaptive protection of the brain.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-280 g. The animals were divided into 5 groups (1 control group and 4 experimental groups). The animals of experimental group 1 received an NO donor. Group 2 animals were adapted to hypoxia. AD was modeled in group 3 rats. Group 4 comprised hypoxia-adapted rats with AD.

The formation of NO stores in rat cerebral vessels was induced by administration of an NO donor (DNIC) 3 h before the start of the experiment [4].

Adaptation to hypobaric hypoxia was carried out in an altitude chamber by "elevation" to an altitude of 4000 m above sea level (4 h per day, 14 days). The last adaptation session was performed 24 h before AD modeling. AD was induced by bilateral stereotactic administration of the neurotoxic peptide fragment (25-35) of β-amyloid (Aβ) (2 μl Aβ (25-35) solution in a concentration of 0.4×10^{-9} M) into the *n. basalis magnocellularis*. The study was performed 30 h after Aβ administration.

The rats were anesthetized with chloral hydrate. A hole was drilled in the parietal region to get access to the parietal cortex. Local cerebral blood flow (LCBF) was continuously recorded using an ALF-21 laser Doppler flowmeter. Endothelium-dependent dilation of cerebral vessels was studied after stabilization of LCBF (increase in response to injection of 10^{-5} M acetylcholine into the carotid artery). Variations in LCBF were expressed in percents of the basal level.

NO stores were detected by the method based on studying the vascular response to N-acetylcysteine (N-AC). This compound destroys NO stores releasing vasoactive products [3,4]. The rats were pretreated with NO synthase inhibitor N^{ω}-nitro-L-arginine (L-NNA, 50 mg/kg intraperitoneally) to exclude the influence of *de novo* synthesized NO on LCBF. N-AC at a concentration 10^{-3} M was injected into the carotid artery toward the head after 20 min. The increase in LCBF was expressed in percents of resting LCBF.

The results were analyzed by Student's *t* test. The data are presented as $M\pm SEM$.

RESULTS

The method of laser Doppler flowmetry is extensively used in LCBF monitoring to study the NO-depended response of cerebral vessels, including endothelium-dependent vasodilation to acetylcholine and endothelium-independent reaction to NO donors [10]. This method adequately reflects relative changes in LCBF [7]. Previous validation studies revealed a linear dependence between the values of LCBF estimated with labeled microspheres and laser Doppler flowmetry [11]. We used this method for evaluation of the endothelium-dependent vasodilation and NO stores in cerebral vessels.

Basal LCBF in rats pretreated with DNIC was much higher compared to the control (45.6±6.3 and 25.7±52.0 arb. units, respectively, $p<0.05$). Endothelium-dependent vasodilation did not differ in control and DNIC-treated rats (Fig. 1). NO stores were not detected in control animals (Fig. 2). It can be hypothesized that NO stores in cerebral vessels are absent under basal conditions. Otherwise, the volume of NO stores is beyond the lower limit of

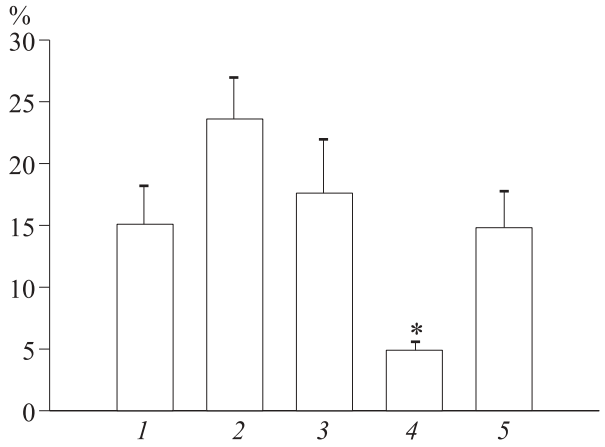

Fig. 1. Effect of dinitrosyl iron complex (DNIC), adaptation to hypoxia, treatment with Aβ (25-35), and Aβ (25-35) administration after preadaptation to hypoxia on the formation of NO storage in cerebral vessels. Ordinate: increase in cerebral blood flow in response to N-acetylcysteine (% of the basal level). Here and on Fig. 2: 1) control; 2) DNIC; 3) adaptation; 4) Aβ; 5) adaptation+Aβ. *p<0.05 compared to the control.

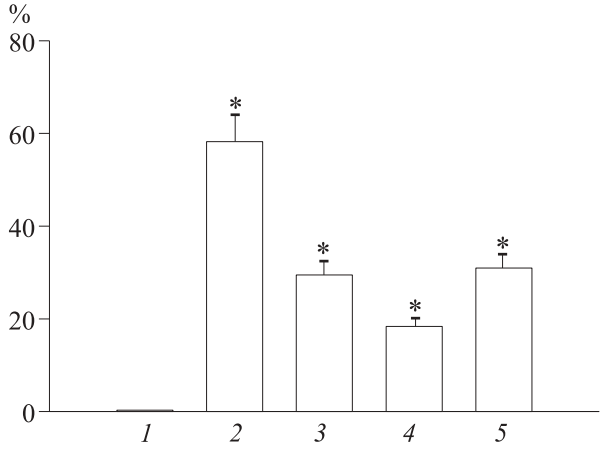

Fig. 2. Effect of dinitrosyl iron complex (DNIC), adaptation to hypoxia, treatment with Aβ (25-35), and Aβ (25-35) administration after preadaptation to hypoxia on the formation of NO stores in cerebral vessels. Ordinate: increase in cerebral blood flow in response to N-acetylcysteine (% of the basal level).

method sensitivity. The latter assumption is most likely, since NO stores in the control basilar artery were revealed using a highly sensitive myograph. Administration of N-AC to DNIC-treated rats caused a transient increase in LCBF by 58.2±18.3%. These changes reflected the presence of NO stores in the vascular wall.

Previous studies showed that NO depots in the vascular walls play a protective role in cardiovascular diseases (*e.g.*, hypertension and hyperthermia) [2]. For example, electron paramagnetic resonance study revealed NO stores in the brain [3] and isolated basilar artery. However, functional role of NO stores in cerebral vessels remained unknown. We used an experimental approach demonstrating the presence of NO stores in the wall of isolated vessels and whole organism *in vivo* [3]. This method is based on physiological assessment of the vascular response to NO release during store destruction. The method allows us not only to reveal NO stores in cerebral vessels, but also to evaluate the influence of this compound on LCBF.

Endothelium-dependent dilation of cerebral vessels in rats 4-fold decreased after Aβ administration (4.8±1.7 *vs.* 15.3±1.2%, $p<0.05$). Our results are consistent with published data that experimental AD is accompanied by endothelial dysfunction of cerebral vessels [10]. Basal LCBF significantly increased after adaptation to hypoxia. It should be emphasized that NO donor DNIC induced a similar increase in LCBF. It can be assumed that the increase in LCBF under both conditions is related to increased NO concentration. LCBF in rats preadapted to hypoxia also increased after Aβ administration. These changes probably prevent cerebral hypoperfusion. Adaptation had no effect on endothelium-dependent dilation, but completely prevented Aβ-induced dysfunction.

N-AC increased LCBF in rats adapted to hypoxia (by 29.5±10.2%, Fig. 2), which reflects the release of NO from stores [2]. Our findings are consistent with published data that adaptation to hypoxia is accompanied by the formation and progressive increase in the volume of NO stores in other vessels [2]. NO stores were also found in Aβ-treated rats. However, the volume of NO stores in these animals was relatively low. Overproduction of NO induced by Aβ was probably followed by storage of excess NO. The volume of NO store in rats adapted to hypoxia before Aβ administration was much higher compared to unadapted Aβ-treated animals.

Aβ plays an important role in the pathogenesis of AD. This compound initiates various neurotoxic mechanisms, including excitotoxicity, impairment of Ca^{2+} homeostasis, free radical processes, NO overproduction, and neuroinflammation [5,12]. NO overproduction is probably associated with hyperactivation of nNOS after long-term stimulation of glutamate receptors. These changes can also be related to hyperactivation of iNOS and induction of this isoform in microglial cells and astrocytes under the influence of cytokines. NO directly induces nitrosylation, which is followed by dysfunction of major proteins with iron-sulfur clusters and thiol residues. Moreover, NO inhibits key enzymes of the Krebs cycle, mitochondrial respiratory chain, and Ca^{2+} metabolism and produces damage to DNA [5].

NO plays a protective role in AD. A permanent decrease in the expression of cerebrovascular eNOS and inhibition of NO production contribute to vascular dysfunction and cerebral hypoperfusion. It is related to impairment of vascular responses and change in the release of metabolites and toxins from the extracellular space. Regional metabolic dysfunction is followed by cognitive disorders and progressive neurodegeneration [12].

Endothelial dysfunction of cerebral vessels plays an important role in impairment of LCBF autoregulation, which is observed in Aβ-treated animals and transgenic animals with Aβ overexpression. A positive correlation was revealed between the severity of endothelial dysfunction and Aβ concentration in the brain [10]. The decrease in endothelium-dependent dilation impairs the reaction of cerebral vessels to transmural pressure drop. These changes make the brain highly susceptible to blood pressure variations even within the normal range and aggravate damage induced by ischemia and occlusion of cerebral vessels [13]. It should be emphasized that Aβ-induced endothelial dysfunction precedes neuro-

degenerative changes in the brain [10] and, therefore, serves as an important pathophysiological mechanism of AD.

Little is known about the possibility of preventing neurodegenerative processes in the brain by adaptation to hypoxia. Previous studies showed that adaptation to hypoxia prevents behavioral disorders associated with neurodegenerative processes during experimental Parkinson's disease [6]. We showed that adaptation to hypoxia improves cognitive function in rats and prevents the decrease in the concentration of nitrites and nitrates in the plasma induced by Aβ [1].

Prolonged moderate stimulation of NO synthesis by endothelial NO synthase during adaptation to hypoxia probably underlies prevention of endothelial dysfunction and blood pressure elevation. Complexes of excess NO are formed during adaptation and accumulated in the vascular wall. Besides this, the ability of vessels to accumulate NO increases under conditions of adaptation to high concentration of NO. This mechanism contributes to vascular protection under conditions of NO overproduction [9].

We showed for the first time that adaptation to hypoxia has a dual protective effect during disturbances in cerebral blood flow induced by a neurotoxic peptide Aβ. This adaptation prevents endothelial dysfunction of cerebral vessels, provides the adaptive response to NO overproduction, and increases binding of excess NO with the formation of stable complexes. Our results indicate that nonpharmacological adaptive prevention of disturbances in cerebral blood flow holds much promise in the therapy of patients with AD.

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