GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Role of Nitric Oxide in Prevention of Cognitive Disorders in Neurodegenerative Brain Injuries in Rats

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NO synthesis disturbances play an important role in the development of neurodegenerative damage in Alzheimer disease. We previously showed that adaptation to intermittent hypobaric hypoxia prevents cognitive disturbances in rats with experimental Alzheimer disease [6]. Here we evaluated the role of NO in cognitive disorders and development of adaptive protection during experimental Alzheimer disease. Adaptation to hypoxia in rats was performed in a hypobaric pressure chamber at a simulated altitude of 4000 m (4 h per day for 14 days). Alzheimer disease was simulated by bilateral injections of a toxic fragment of β-amyloid (25-35) into *n. basalis magnocellularis*. For evaluation of the role of NO in the development and prevention of memory disorders, the rats received intraperitoneally either NO-synthase inhibitor N^{ω}-nitro-L-arginin (L-NNA, 20 mg/kg, every other day for 14 days) or NO-donor dinitrosyl iron complex (200 μg/kg daily for 14 days). NO-synthase inhibitor potentiated the damaging effect of β-amyloid, abolished the protective effect of adaptation to hypoxia, and produced memory disorders in rats similar to those observed during experimental Alzheimer disease. In contrast, the increase in NO level in the body provided by injections of the NO-donor produced a protective effect against memory disorders caused by β-amyloid similar to that induced by adaptation to hypoxia. We concluded that reduced NO production in the organism plays an important role in the development of cognitive disorders produced by injections of β-amyloid, while prevention of NO deficit by administration of NO-donors or non-pharmacological stimulation of NO synthesis can provide a protective effect in experimental Alzheimer disease.

Key Words: *nitrogen oxide, adaptation to hypoxia, Alzheimer disease, nitrogen oxide donor, NO-synthase inhibitor*

Overproduction and deficiency of NO play an important role in the development of neurodegenerative damage in Alzheimer disease (AD). Excess of NO released by astrocytes and microglia produces a direct neurotoxic effect, whereas deficit of endothelial NO leads to dysfunction of vascular endothelium and hypoperfusion of the brain. Hence, the search for new drugs protecting the brain from neurodegenerative damage via modulation of NO synthesis is an urgent problem.

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Much recent attention is paid to factors improving the adaptive potential of the organism, mobilizing endogenous protective systems, and thus preventing damage to brain cells in Alzheimer disease. The efficiency of adaptation to diet, physical exercises, and mental exercises is proven. The protective effect of these types of adaptation is based on the expression of cytoprotective stress-proteins HSP, limitation of oxidative stress, inhibition of apoptosis, and even stimulation of neurogenesis in adult human brain [2].

Organism's adaptation to environmental factors such as special diets, physical exercises, stress, heat, and especially intermittent hypoxia is associated with long-term stimulation of NO synthesis. This process is probably responsible for effective therapeutic and prophylactic adaptation in cardiovascular diseases associated with endothelium dysfunction. At the same time, it was shown that this adaptation could prevent not only deficiency, but also overproduction of NO in myocardial infarction and different types of shock [14].

We previously showed that adaptation to intermittent hypobaric hypoxia prevented cognitive disorders in experimental AD [5]; moreover, this protective effect was followed by normalization of the function of brain vessel endothelium [4]. Therefore, for evaluation of possible mechanisms underlying the preventive effect of adaptation to hypoxia in experimental AD we studied the role of NO in the formation of adaptive protection.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighting 320±50 g from the vivarium of Institute of General Pathology and Pathophysiology. Each group consisted of 10 rats.

AD was modeled by bilateral stereotaxic injection of the neurotoxic fragment of β-amyloid (25-35) into *n. basalis magnocellularis* (2 ml of 0.4×10^{-9} M β-amyloid (25-35) solution). The animals were taken into experiment 30 days after β-amyloid injection. Previous experiment showed that saline injected instead of β-amyloid produced no damaging effect [6], therefore this control was not used in the present study.

Adaptation to hypobaric hypoxia was performed in a pressure chamber at a simulated altitude of 4000 m above sea level, 4 h per day for 14 days. The last session was carried out 24 h before AD modeling.

The degree of neurodegenerative damage was evaluated by memory impairment in conditioned passive avoidance (CPA) test 14 days after β-amyloid injection. The rats were placed on an illuminated area and the latency of transition into the dark chamber (L1) was recorded over 3 minutes. After entering the dark chamber, the rat received inescapable electric shock through the floor (0.7 mA current, 12 consecutive 1-sec stimuli with 2-sec intervals). The test was repeated after 24 h. The rat was placed again into the experimental setup and the latency transition into the dark chamber (L2) was recorded over 3 min. If memory was safe, the animal remembered about painful stimulation in the dark chamber and did not entered it or entered much later, i.e. the latency of entering the dark chamber was longer; thus, greater difference between L2 and L1 (DL) indicated better memory retention.

For evaluation of the role of NO in the development and prevention of memory disorders, the rats received intraperitoneally either 20 mg/kg NO-synthase inhibitor N^{ω}-nitro-L-arginin (L-NNA, Sigma) every other day for 14 days or 200 μg/kg dinitrosyl iron complex (DNIC, NO-donor) every day for 14 days. For evaluation of the role of NO in protective effects of adaptation to hypoxia, inhibitor L-NNA or DNIC were administered during the course of adaptation.

NO production in the organism was measured spectrophotometrically from the total plasma concentration of stable NO metabolites, nitrites and nitrates, using Griss reaction. Nitrates reduction to nitrites was performed using Nitralyser kits (World Precision Instruments Inc.).

The data were analyzed using paired Wilcoxon *T* test (comparison of L1 and L2 and calculation of DL in conditioned passive avoidance test for each animal) and nonparametric Mann—Whitney *U* test (comparison of conditioned passive avoidance performance in different series). Plasma levels of nitrates and nitrites were compared using Student's *t* test.

RESULTS

Results of passive avoidance conditioning showed that 89% rats remembered electric shock, what was seen from increased latency of enter into the dark part of the experimental chamber. After β-amyloid injection, the differences between L2 and L1 were significantly lower than in the control group, which attested to memory impairment (Fig. 1, *a*, *b*). The percent of rats remembering electric shock decreased to 75% ($p<0.05$). Adaptation to hypoxia per se had practically no effect on memorization, but DL after β-amyloid administration was greater in adapted rats than in nonadapted animals, *i.e.* adaptation prevented memory impairment induced by β-amyloid administration.

The course of L-NNA significantly impaired memorizing in rats (Fig. 1, *a*). Administration β-amyloid after L-NNA treatment aggravated memory disturbances induced by β-amyloid. L-NNA decreased DL from 135.8 sec (control) to 122 sec; in rats receiving β-amyloid L-NNA decreased DL from 58.1 sec to 38.8 sec. Administration of L-NNA during adaptation to hypoxia abolished its protective effect.

Course treatment with DNIC per se had no significant effect on passive avoidance performance (Fig, 1, *b*). Administration of β-amyloid after DNIC treatment only insignificantly impaired passive avoidance performance. In adapted rats receiving simultaneously DNIC, the protective effect against β-amyloid-induced damage was similar to that observed without DNIC treatment. In other words, DNIC administration reproduced the protective effect of adaptation, but did not potentiated it.

The development of experimental AD was accompanied by a decrease in the content of nitrites and nitrates by 63% ($p<0.05$; Fig. 2). Preliminary adaptation to hypoxia moderately stimulated NO synthesis and significantly reduced its decrease after β-amyloid injection.

Course treatment with L-NNA decreased plasma level of nitrites and nitrates from 52.4±3.9 to 32.4±9.1 μM (Fig. 2, *a*). Administration of β-amyloid after L-NNA treatment further decreased the content of nitrites and nitrates to 12.8±3.2 μM. Administration of L-NNA during adaptation to hypoxia abolished the adaptive increase in the content of nitrites and nitrates: this parameter was similar in adapted and nonadapted rats before and after β-amyloid injection, respectively.

Course treatment with DNIC increased plasma level of nitrites and nitrates in all groups (Fig. 2, *b*). Administration of β-amyloid after DNIC treatment did not decrease the level of nitrites and nitrates. DNIC administration during adaptation induced the same increase in this parameter as in the control, while after administration of β-amyloid to adapted animals it only slightly decreased.

Principal finding of this study is that NO synthase inhibitor L-NNA aggravated the damaging effect of β-amyloid, abolished the protective effect of adaptation to hypoxia and, moreover, induced memory disorders in rats similar to those occurring in experimental AD. In contrast, the increase in NO level after administration of NO donor DNIC protected the organism from memory impairment induced by β-amyloid administration similar to that provided by adaptation to hypoxia.

The role of NO in DA development is ambiguous. On the one hand, accumulation of β-amyloid in the brain induces NO overproduction in microglia and astrocytes [7]. The neurotoxic effect of NO excess is mediated by mitochondrial dysfunction and ATP depletion leading to neuron apoptosis [13]. On the other hand, β-amyloid inhibits activity of endothelial NO synthase, thus leading to deficiency of endothelial NO and endothelial dysfunction not only in cerebral, but also in peripheral vessels [11]. Decreased plasma level of NO metabolites is a marker of NO deficiency [10], which was observed also in our experiments. NO deficiency aggravates AD-related disorders mediating brain hypoperfusion. That is why NO can be regarded as not only a damaging, but also a protective factor during AD.

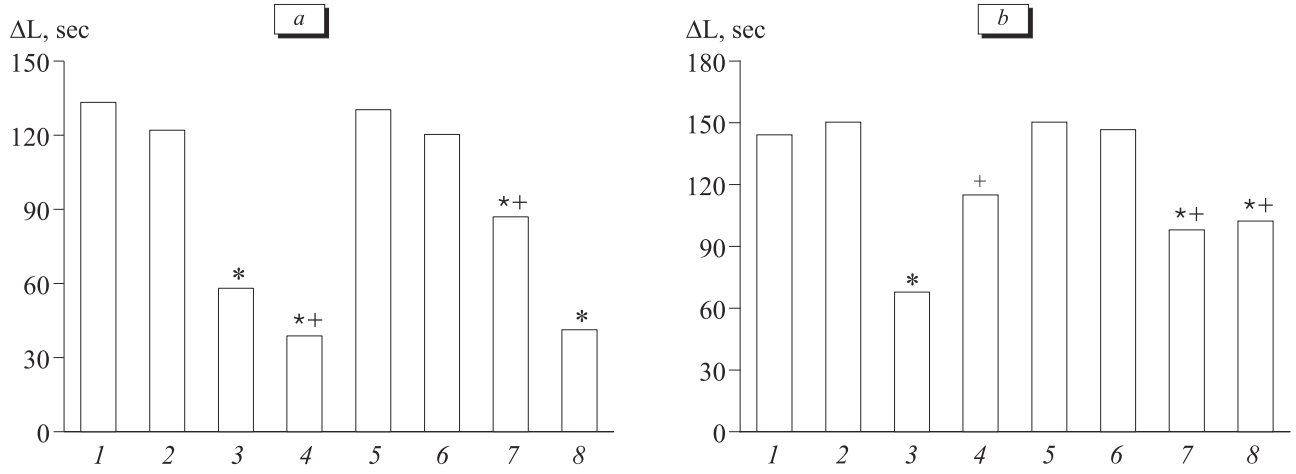

Fig. 1. Effects of preliminary adaptation to hypoxia, NO-synthase inhibitor L-NNA (*a*) and NO-donor DNIC (*b*) on β-amyloid-induced memory disorders in rats. On Fig. 1, *a* and Fig 2, *a*: *1*) control; *2*) L-NNA; *3*) β-amyloid; *4*) L-NNA+β-amyloid; *5*) adaptation; *6*) adaptation+L-NNA; *7*) adaptation+β-amyloid; 8) adaptation+L-NNA+β-amyloid. On Fig. 1, *b* and Fig. 2, *b*; *1*) control; *2*) DNIC; *3*) β-amyloid; *4*) DNIC+β-amyloid; *5*) adaptation; *6*) adaptation+DNIC; *7*) adaptation+β-amyloid; 8) adaptation+DNIC+β-amyloid. Here and on Fig. 2: $p<0.05$ compared to: *control, $^{+}$β-amyloid administration.

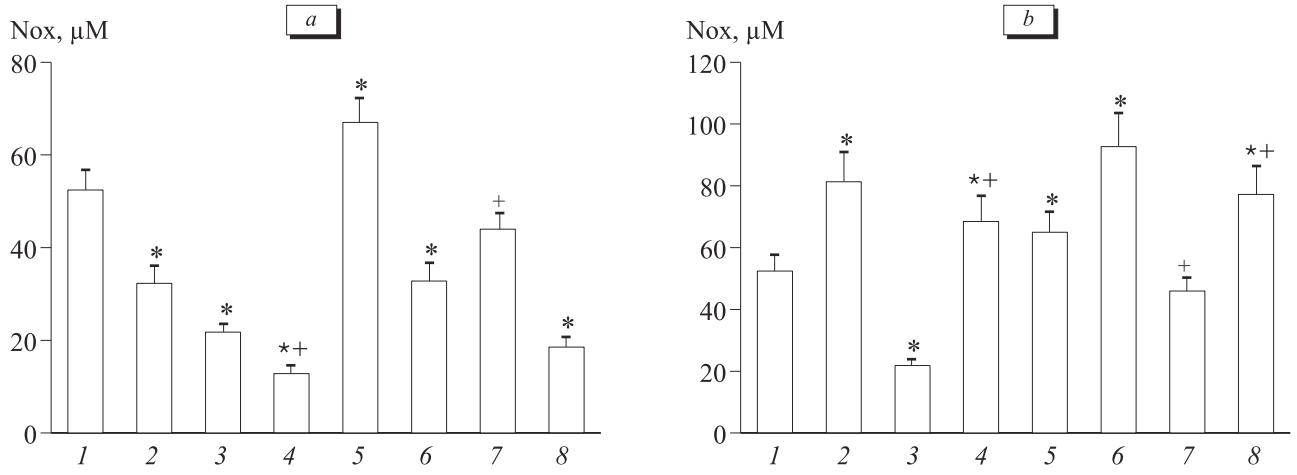

Fig. 2. Effects of preliminary adaptation to hypoxia, NO-synthase inhibitor L-NNA (*a*) and NO-donor DNIC (*b*) on the content of sable NO metabolites (nitrites and nitrates) in rats after injection of β-amyloid. Total plasma concentration of nitrites and nitrates is shown.

Astrocytes and epithelial cells intensively producing NO are better protected against its toxic effects than neuronal cell lines characterized by weak NO production [12]. The level of nitrates in the cerebrospinal fluid negatively correlates with the degree of mental disorders in AD patients [15]. In our experiments, rats receiving DNIC were more tolerant to neurodegenerative damage, while rats with decreased NO level (receiving L-NNA) were more vulnerable to β-amyloid-induced damage than control animals. These results are consistent with published reports demonstrating that AD proceeds significantly more severe course and is accompanied by more pronounced pathological alterations in endothelial NO synthase gene knockout rats [9], and with our previous observations that August rats characterized by higher level of NO production are better protected against neurodegenerative damages than Wistar rats [6].

Preliminary adaptation to intermittent hypoxia effectively prevents behavioral disorders [5] and endothelium dysfunction of brain vessels in rats with experimental AD [4]. This study proved that this protective effect, at least partially, is mediated by long-term nonpharmacological stimulation of NO synthesis. This assumption is confirmed by the fact that NO synthase inhibitor L-NNA prevents the development of adaptative protection, while NO donor DNIC reproduces it.

Adaptation to intermittent hypoxia stimulating the synthesis of endothelial NO effectively prevents NO overproduction and limits its adverse consequences [14]. Adaptative protection from overproduction and deficiency of NO can be mediated by two mechanisms. First, NO overproduction is limited by NO itself by the negative feedback mechanism [8]. Hence, preliminary intensification of NO synthesis induced by adaptation can play a role in prevention of NO overproduction. Adaptation to hypoxia promotes binding of NO excess into complexes forming NO stores in the vascular wall [1]. The formation of these stores seems to be an adaptive reaction aimed at protection of the organism against toxic effects of NO excess. At the same time, NO stores can act as supplementary nonenzymatic source of free NO, which to a certain extent can compensate for its deficiency [3]. Our previously experiments proved the role of NO stores in the protective effects of adaptation to hypoxia against toxic action of β-amyloid on brain vessels [4].

Thus, the decrease in NO production plays an important role in the development of cognitive disorders caused by β-amyloid. Prevention of NO deficiency by NO donors and nonpharmacological stimulation of NO synthesis can produce a protective effect in experimental AD.

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