Antioxidant and Neuroprotective Properties of N-Docosahexaenoyl Dopamine

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N-Docosahexaenoyl dopamine exhibited antioxidant activity in the test with a stable oxygen radical galvinoxyl. This compound produced a dose-dependent protective effect on cultured granular cells from rat cerebellum under conditions of oxidative stress. N-Docosahexaenoyl dopamine decelerated the development of symptoms of Parkinson's disease in mice receiving neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Key Words: *N-docosahexaenoyl dopamine; N-acyldopamines; antioxidants; neuroprotection; Parkinson's disease*

Dopamine derivatives acylated with fatty acids belong to a recently discovered family of endogenous neurolipins with a wide range of effects. They modulate activity of the endocannabinoid and endovanilloid systems in the brain [4]. N-Acyldopamins can be considered as a transport form of this biogenic amine capable of crossing the blood-brain barrier and producing the dopaminergic effect. This property of acyldopamins can be used for normalization of dopaminergic transmission in Parkinson's disease. N-Docosahexaenoyl dopamine (DHX-DA) increases the content of dopamine and its metabolites in the brain tissue in rodents [8]. Acylated form of biosynthetic dopamine precursor N-linolenoyl tyrosine also increases brain dopamine concentrations and produces an antiparkinsonic effect in rats with experimental Parkinson's disease [5].

Oxidative stress is a factor causing progressive neuronal death during Parkinson's disease [9]. Treatment with antioxidants can decelerate the development of symptoms of this disease. Various compounds containing catechol fragments are potent antioxidants [2,6]. N-Acyldopamine molecules also contain catechol residue. However, antioxidant potency of these compounds remains unknown.

Here we studied antioxidant properties of DHX-DA belonging to the family of N-acyldopamines (Fig. 1, *a*) [3]. The neuroprotective effect of DHX-DA was evaluated in cultured granular cells from rat cerebellum after oxidative stress. The influence of DHX-DA on symptoms of Parkinson's disease was assayed on mice.

MATERIALS AND METHODS

Antioxidant activity was measured in the test with galvinoxyl (GO) [6]. GO reduction with hydrogen proton-donating compounds is followed by a decrease in light absorption at 428 nm (Fig. 1, *b*, *d*). In the reaction mixture containing excess GO, all proton-donating chemical groups of DHX-DA can react with the radical (Fig. 1, *c*). The number of chemical groups in the molecule capable of reducing the radical can be estimated from the molar extinction coefficient of GO, concentration of the reactive compound, and decrease in absorption. Equivalent volumes of GO and antioxidants were

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mixed in ethanol to initiate the reaction. The final concentration of GO was 10 μM. The concentration of DHX-DA and α-tocopherol varied from 0.1 to 2.0 μM. The decrease in absorption of GO at 428 nm was recorded after 20-min incubation using an Ultraspec spectrophotometer (LKB).

Granular cells of rat cerebellum were isolated and cultured [1]. To produce experimental oxidative stress, the neurons were incubated in a medium containing H_2O_2 (1 mM) and DHX-DA or α-tocopherol (0.1-10.0 μM) at room temperature for 30 min. Cell viability was estimated by the ability to reduce dimethylthiazole diphenyl tetrazolium bromide (MTT test) into water-insoluble formazan. The cells were dissolved in dimethylsulfoxide. Formazan absorption (A) was measured on an Ultraspec spectrophotometer (LKB) at 570 and 620 nm. The relative number of viable cells was calculated by the equation: A_{570}-A_{620}.

Parkinson's disease in CBA mice was induced by intraperitoneal injection of neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The symptoms of Parkinson's disease in MPTP-treated mice (*e.g.*, decrease in locomotor activity) develop due to depletion of catecholamine reserves in brain neurons [7]. Neurotoxin-induced changes in mouse behavior were studied in 10-min open-field test. There were 2 schemes of neurotoxin treatment. In acute experiments, the mice were injected with MPTP in a dose of 30 mg/kg. After 90 min some animals received physiological saline, while others were treated with DHX-DA (5 mg/kg). Spontaneous locomotor activity in the open field was studied after 24 h. In chronic experiments, the mice received MPTP in a daily dose of 30 mg/kg for 10 days. Some animals received physiological saline, while others were treated with DHX-DA (10 mg/kg) 1 h after the last injection on day 10. Spontaneous locomotor activity in the open field was studied after 24 h.

RESULTS

The molar extinction coefficient of GO in ethanol was 1.2×10^5. Treatment with DHX-DA (0.1-1.0 μM) was followed by a dose-dependent and linear decrease in GO absorption. The stoichiometric index for this compound was calculated as follows: n=tgα/ε1, where tgα is the slope ratio; and ε is the molar extinction coefficient of GO in ethanol (λ=1 cm). The value of n for DHX-DA was 2.01. The index approaching 2 indicates that the molecule contains 2 groups capable of reducing GO. Similar results were obtained in experiments with the lipid antioxidant α-tocopherol. The stoichiometric index for α-tocopherol was 1.3, which is consistent with published data [6]. We conclude that under these experimental conditions DHX-DA is a more potent blocker of oxygen radicals compared to α-tocopherol (by 2 times). It can be hypothesized that antioxidant activity of DHX-DA in this test system is associated with the presence of hydroxyl groups.

Antioxidant properties of DHX-DA were also demonstrates in experiments with cultured cells. Incubation of neurons with 1 mM H_2O_2 was followed by a 50% decrease in the number of viable cells. Light microscopy of cultures after 30-min incubation with H_2O_2 revealed a considerable number of swollen and destructed cells. In the presence of DHX-DA morphological characteristics of neurons did not differ from those typical of intact cells. Biochemical tests showed that DHX-DA produces a dose-dependent protective effect. This effect was most significant under the influence of DHX-DA in a concentration of 10 μM (80% viable cells, Fig. 2). Natural antioxidant α-tocopherol had no protective

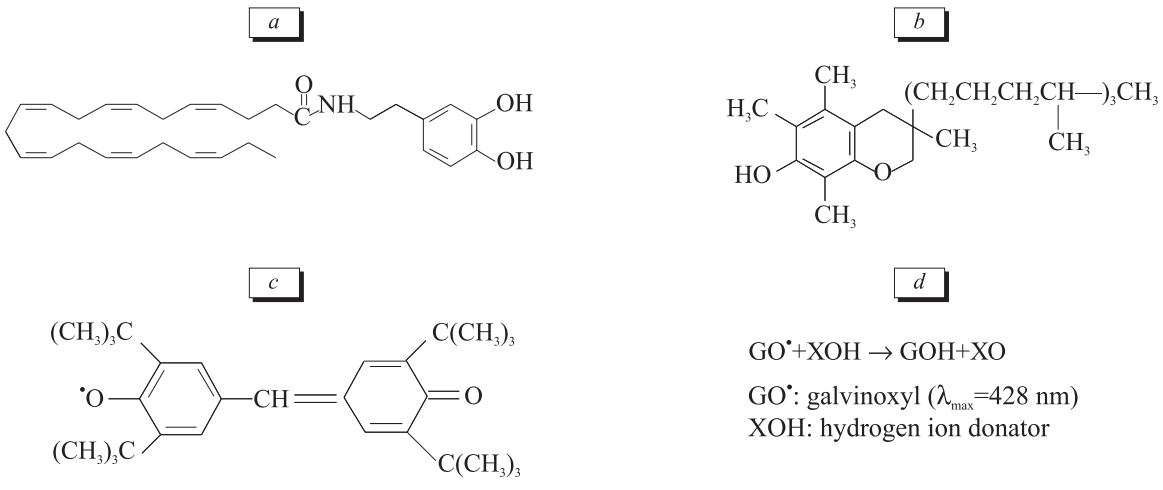

Fig. 1. Structure of test compounds. DHX-DA (*a*); α-tocopherol (*b*); scheme of GO reduction with antioxidants (*c*).

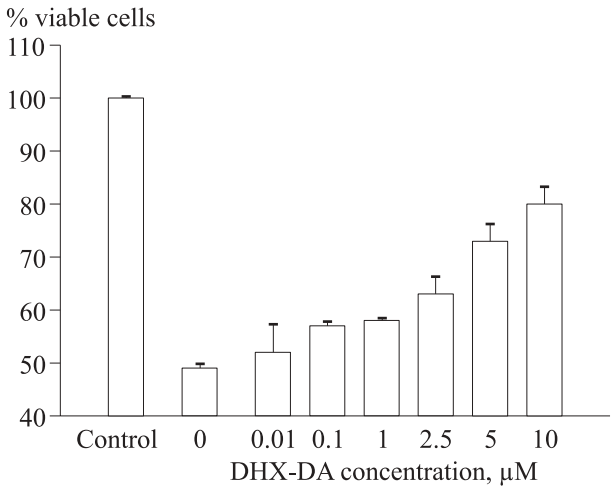

Fig. 2. Protective effect of DHX-DA on the model of oxidative stress. Viability in the MTT test.

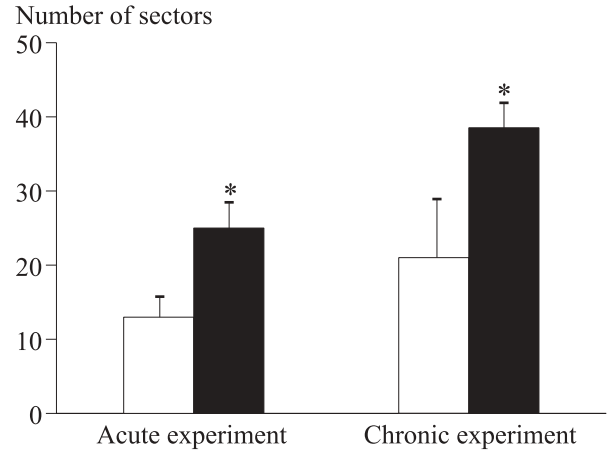

Fig. 3. Effect of DHX-DA on the decrease in locomotor activity of mice induced by acute and chronic administration of MPTP. Light bars, locomotor activity after MPTP administration; dark bars, treatment with MPTP and DHX-DA. *p<0.1 compared to the parameter without DHX-DA.

effect under these experimental conditions. These data suggest that DHX-DA will produce a neuroprotective effect under various conditions of overproduction of reactive oxygen species.

Intraperitoneal injection of MPTP was followed by a significant (by 70%) and long-lasting decrease in locomotor activity of animals in the open-field test. Acute experiments showed that intraperitoneal injection of DHX-DA (5 mg/kg) 90 min after MPTP treatment increases locomotor activity of animals by 1.5 times. These changes were revealed on the next day after injection. Single administration of DHX-DA (10 mg/kg) after 10-day treatment with MPTP increased locomotor activity of animals by 1.8 times (Fig. 3). The observed effects are associated with partial recovery of function of the dopamine system under the influence of dopamine from DHX-DA.

The development of Parkinson's disease is related to death of dopaminergic neurons in the substantia nigra. Neuronal death is partly associated with oxidative stress [11]. Pharmacological agents that inhibit overproduction of reactive oxygen species and maintain the required level of dopamine in the brain hold promise for preventing the symptoms of Parkinson's disease. We showed for the first time that DHX-DA belonging to the family of N-acyldopamines exhibits antioxidant and neuroprotective properties. This compound can be used for the correction of neurodegenerative changes in the central nervous system (*e.g.*, during Parkinson's disease).

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