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The Role of MARK ERK1/2 and p38 in Regulation of Functions of Neural Stem Cells and Neuroglia under Conditions of β-Amyloid-Induced Neurodegeneration

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The role of ERK1/2 and p38 in the realization of the growth potential of neural stem cells and secretion of neurotrophic growth factors by glial cells was studied using *in vitro* model of β-amyloid-induced neurodegeneration. It was shown that amyloid-β fragment 25-35 significantly inhibits the cell cycle progression of neural stem cells against the background of stimulation of their differentiation and reduced production of growth factors by neuroglia. The inhibitory role of ERK1/2 and p38 in relation to the proliferative activity of neural stem cells and the secretory activity of glial elements was revealed. ERK1/2 and p38 inhibitors increased proliferation of progenitor cells of the nervous tissue and reduced the intensity of their specialization, as well as stimulated production of growth factors by neuroglial cells under conditions of simulated β-amyloid-induced neurodegeneration.

Key Words: *Alzheimer's disease; neural stem cells; neuroglia; intracellular signal transduction; mitogen-activated protein kinases*

Alzheimer's disease (AD) is the most common form of dementia in the elderly. In recent years, a dramatic increase in the incidence of AD and a significant decrease of the debut age are observed [2,8]. AD is characterized by a progressive impairment of cognitive functions and the loss of practical skills and self-care abilities, which ultimately leads to death due to causes unrelated to the underlying disease (infections, food aspiration, *etc*.). The development of these disorders occurs against the background of decompensation of adaptive mechanisms in various compartments of the nervous tissue [7,15]. The synthesis of neurotoxic β-amyloid peptides and the formation of neurofibrillary tangles are accompanied by a significant reorganization of the CNS and the formation of a qualitatively new pathogenic pattern of activity of individual brain structures [1,5].

Currently used drug therapy is based on the impact on the three main putative causes of AD: cholinergic, amyloid, and tau-protein hypotheses [6,8]. However, the drugs developed within the framework of these concepts are little effective [3], which indicates the relative failure of the cholinergic, amyloid, and tau-hypotheses, at least as the trigger mechanisms for the formation of the pathology. However, the neurotoxic effect of β-amyloid (Aβ) and its critically important pathogenetic role in AD course are beyond doubt [9].

The death of neurons and destruction of intercellular contacts (synapses) under the influence of Aβ are observed against the background of the loss of the ability for balanced neuro-, neurito-, and synaptogenesis in the nervous tissue [2]. Therefore, stimulation of neurogenesis coordination with pharmacological agents, regulators of regeneration-competent cells of various classes (including neural stem cells (NSC) and neuroglial elements), it a promising approach in the search for AD therapy options [11-15]. This approach

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also looks promising if the data on the etiological role of somatic mutations in nerve cells (including "transcriptional mutations" in neurons [3,4]) in the development of AD are confirmed. It is possible that activation of the "deep reserve" regeneration mechanisms ("dormant" NSC) can lead to the formation of nerve cells that do not have genetic and/or biochemical "defects" acquired in this way.

It is rational to search for ways to solve this problem within the framework of the "Strategy of Targeted Pharmacological Regulation of Intracellular Signal Transduction in Regeneration-Competent Cells" [10,11,13-15]. The fundamental stage in the development of this direction is evaluation of the role of individual elements of intracellular signal transduction in the regulation of the functions of various types of regeneration-competent cells. These studies will make it possible to determine the optimal pharmacological targets for potential modifiers of the activity/expression of signaling molecules [9-15]. It is known that MAPK signaling is involved in the regulation of proliferation and differentiation of progenitor cells, as well as in the production of cytokines by glial elements [12]. However, there is no detailed understanding of the significance of individual directions of the MAPK signaling pathways (including pathways mediated by activation of protein kinases ERK1/2 and p38) in regeneration-competent cells exposed to Aβ.

Our aim was to reveal the involvement of MAPK ERK1/2 and p38 in the growth potential of NSC and secretion of neurotrophic growth factors by glial elements of the nervous tissue under conditions of modeled Aβ-induced neurodegeneration *in vitro*.

MATERIALS AND METHODS

The studies were carried out on C57BL/6 mice (*n*=30, age 2-2.5 months, body weight 20-22 g), the 1st category (conventional mice) obtained from the Department of Biomodeling of the E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine. The study was conducted in compliance with the principles of humane treatment of experimental animals and were approved by the Local Ethical Committee.

Amyloid β Fragment 25-35 (Calbiochem) was used to simulate Aβ-induced neurodegeneration *in vitro*. This neurotoxic agent in a concentration of 1 mM was incubated for 7 days at 37°C, 5% CO_2, and 100% humidity for protein aggregation. Then, $Aβ_{25-35}$ was introduced *in vitro* into the culture medium to a final concentration of 20 μM.

Using cultural methods, we studied the direct effect of MAPK inhibitors ERK1/2 (PD98059) and p38 (SB202190) (Calbiochem) on the realization of the growth potential of NSC and the secretion of growth factors by glial cells *in vitro*. The working concentration of inhibitors was determined in preliminary experiments and was *in vitro* 100 and 10 μM, respectively. Cell cultures without inhibitors of signaling molecules served as controls.

NSC were studied by culturing unfractionated cells of the subventricular zone of the cerebral hemispheres. To this end, cells at a concentration of 10^5/ml were incubated in MACS Neuro Medium (Miltenyi Biotec) for 5 days in a CO_2 incubator at 37°C, 5% CO_2, and 100% air humidity. After incubation, the content of clonogenic cells, in particular NSC (CFU-N, neurospheres containing >100 cells) and committed neural precursors (ClFU-N, neurospheres consisting of 30-100 cells) were counted, their mitotic activity and intensity of specialization of CFU were evaluated. The proliferative activity of progenitor cells was assessed by the hydroxyurea cell suicide method. The intensity of specialization processes (differentiation/maturation) of progenitor elements was determined by calculating the ClFU/CFU ratio (differentiation index) [14,15].

The secretory function of glial cells, *i.e.* total production of growth factors stimulating CFU-N (colony-stimulating activity) was assessed by the effect of conditioned media of 2-day cell cultures of the subventricular region of the cerebral hemispheres (containing Aβ and inhibitors of ERK1/2 or p38) on the level neurosphere formation in the test-system, a culture of intact cells of the subventricular zone (concentration 10^5/ml) in MACS Neuro Medium [12,15].

The results were processed by the method of variation statistics using the Statistica 6.0 software (StatSoft, Inc.) using the nonparametric Mann–Whitney *U* test (mean value of the indicator, the significance of differences in indicators between groups at $p<0.05$).

RESULTS

Addition of the neurotoxic fragment $Aβ_{25-35}$ to the culture of cells of the subventricular zone of the cerebral hemispheres led to a decrease in the level of colony formation. The number of CFU-N was 78.7% of the level observed in the cell culture without Aβ (Fig. 1). These changes reflected a decrease in the rate of NSC division (to 71.5% of the corresponding parameter in the medium without Aβ). However, the number of ClFU-N increased to 141.1% of the initial level under these conditions, which was a natural consequence of an increase in the intensity of NSC specialization. The differentiation index of progenitor cells reached 181.1% of that in the medium without the neurotoxic agent. Moreover, these changes were observed against the background of reduced production of neurotrophic growth factors by glial cells (15.5% of the corresponding parameter in the medium without Aβ) (Fig. 2).

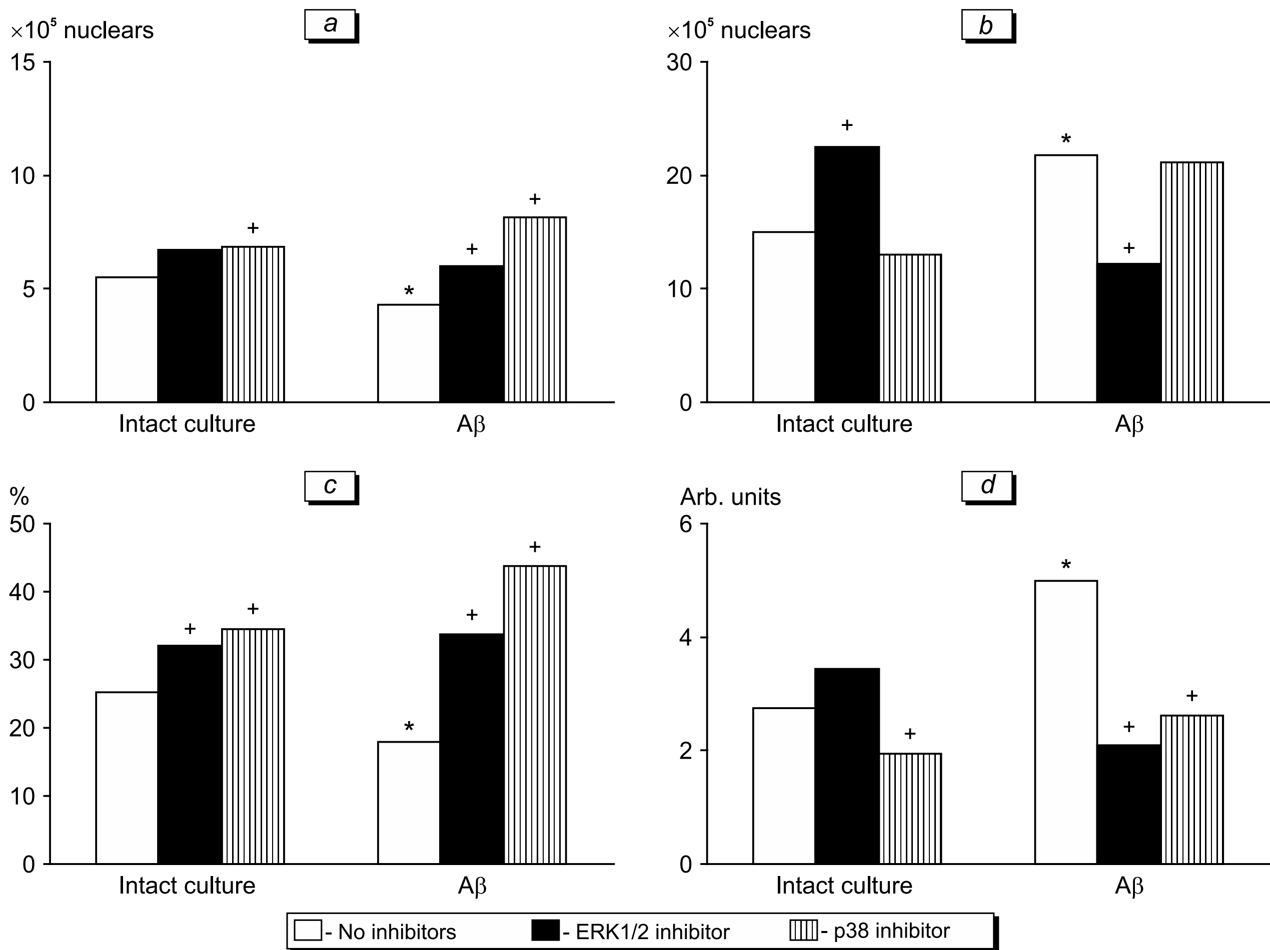

Fig. 1. Number of CFU-N (*a*), ClFU-N (*b*), proportion of S-phase CFU-N (*c*), and intensity of CFU-N differentiation (*d*) in the cell culture of the subventricular zone of the brain of intact C57BL/6 mice incubated without βA (intact culture) and with βA after addition of inhibitors of ERK1/2 and p38. $p<0.05$ in comparison with *intact culture, +culture without inhibitors.

The revealed phenomena correspond to the published data on the disruption of the mitotic activity of the ancestral cells of the nervous tissue under the influence of Aβ [2,6]. At the same time, the results obtained indicate a significant uncoupling of the processes of NSC proliferation and differentiation under conditions of Aβ-induced neurodegeneration. At the same time, it is known that accelerated maturation of progenitor elements can be accompanied by *de novo* development of functionally defective mature cells [5,9]. The detected reaction of neuroglia under the action of Aβ should also be considered ambiguous. The revealed decrease in the colony-stimulating activity of supernatants obtained by culturing neuroglia in the presence of a toxic agent could be associated with both a decrease in the secretion of growth factors and an increase in the production of inhibitors of NSC proliferation by glial cells. These substances include a wide range of proinflammatory cytokines produced primarily by microglial cells [6,12,15]. At the same time, it is believed that pronounced inflammatory reaction in the nervous tissue in AD has a pathogenic significance [6].

The study of the involvement of MAPK-dependent signal transduction pathways in the realization of the growth potential of neural progenitors revealed a number of interesting phenomena. The introduction of ERK1/2 and p38 inhibitors into the culture medium in both cases (in the presence and without Aβ) led to an increase in the level of colony formation and the rate of CFU-N division (Fig. 1, *a*, *c*). The number of CFU-N and their mitotically active forms under conditions of neurodegeneration modeling reached 138.6 and 187.1% with ERK1/2 blockade and 188.7 and 243.5% with p38 inactivation from the corresponding control levels (Fig. 1). Moreover, the values of these indicators under the influence of ERK1/2 and p38 inhibitors were achieved under conditions of modeling Aβ-induced neurodegeneration, values similar to those in the medium without Aβ. That is, the pharmacological agents used completely leveled the negative effect of Aβ on the progression of the NSC cell cycle. In

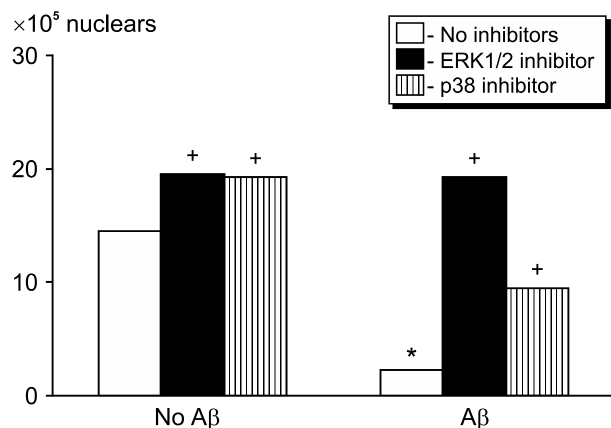

Fig. 2. Effect of conditioned cells of the subventricular zone of the brain of intact C57BL/6 mice on the level of the formation neurospheres in the test system. Conditioned media were obtained after cell culturing in a medium without βA (intact) and with βA and after addition of inhibitors of ERK1/2 and p38. p<0.05 in comparison with *intact culture, +culture without inhibitors.

addition, inhibitors of ERK1/2 and p38 in the presence of Aβ reduced the differentiation index of progenitor cells to that of intact cells (Fig. 1, *d*). Disruption of signal transmission through protein kinases was also accompanied by a significant increase in the colony-stimulating activity of conditioned media of nerve cells during their cultivation both in the presence of Aβ and without it (Fig. 2). Under conditions of modeled neurodegeneration with blockade of ERK1/2 and p38, this parameter increased to 855.6 and 411.1% of the control level (medium with Aβ without inhibitors of signaling molecules), respectively.

In general, the results of studies indicate a pronounced discoordination of the functioning of NSC and a violation of the implementation of the humoral neurotrophic function (aimed at stimulating the progression of the cell cycle [11,12]) by neuroglial cells under the influence of Aβ. The revealed changes (desynchronization of the functions of regeneration-competent cells) can cause the formation of mature cells of the nervous tissue, which have certain functional "defects" [5,9], which are under the influence of phosphorylated tau-proteins and disorders of the cholinergic system in AD [8,9] *in situ* will only be more pronounced and aggravated. At the same time, it was shown that ERK1/2 and p38-dependent signaling play an important role in the development of the discovered mechanisms of disadaptation [9,10,12]. The possibility of conjugation of the processes of proliferation and differentiation of NSC, as well as activation of the compensatory response of neuroglia in Aβ-induced neurodegeneration using selective inhibitors of ERK1/2 and p38, was demonstrated for the first time.

The results indicate the expediency of finding a solution to the problem of neurogenesis disorders in AD in the framework of the "Strategy of Targeted Pharmacological Regulation of Intracellular Signal Transduction in Regeneration-Competent Cells" [10-15]. At the same time, the prospect of developing approaches to stimulate coordinated (full-fledged) neuroregeneration based on ERK1/2 and p38 inhibitors is obvious.

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