Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 13, Issue 5, 2023, 401
https://doi.org/10.33263/BRIAC135.401

Inhibitors of Intracellular Signaling Molecules: New Horizons in Drug Discovery for the Treatment of Alzheimer's Disease

Gleb Nikolaevich Zyuz'kov [1,*], Larisa Arkad`evna Miroshnichenko [1], Larisa Yur`evna Kotlovskaya [1], Alexander Vasil`evich Chayikovskyi [1]

[1] Laboratory of Pathological Physiology and Experimental Therapy, Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia, 3 Lenin Prospect, Tomsk, 634028, Russia
* Correspondence: zgn@pharmso.ru (G.N.Z.);

Scopus Author ID 6508338421
Received: 9.09.2022; Accepted: 7.10.2022; Published: 16.11.2022

Abstract: Alzheimer's disease (AD) is a prime example of a pathology with extremely low treatment efficiency. The relevance of developing new drugs for its treatment is beyond doubt. Modern research in the discovery of therapeutic intervention in some neurodegenerative diseases indicates a high prospect of using intracellular signaling pathways as targets. We studied the effect of selective inhibitors of MEK1, p38α/p38β, NF-κB, JAKs, STAT3, adenylate cyclase, and PKA on the functioning of neural stem cells (NSC) and committed neuronal precursors (NCP) in an *in vitro* model of amyloid-β-induced neurodegeneration. The results of the experiments indicate the formation of fundamentally new patterns of intracellular signaling during the regulation of proliferation and specialization of progenitors under the influence of neurotoxic fragments of β-amyloid. There is a desynchronization of NSC and NCP activities. It was found that the pharmacological regulation of signal transduction in regeneration-competent cells (RCCs) with the help of selective inhibitors of some intracellular molecules leads to the coordination of the pro-regenerative capacity of different types of progenitors. The findings indicate the prospect for the discovery of new drugs with neuroregenerative activity for the treatment of AD based on inhibitors of MEK1, p38α/p38β, NF-κB, STAT3, and PKA. The obtained data expand the horizon of using modifiers of intracellular signaling molecule activity and open new prospects for targeted regulation of intracellular signaling.

Keywords: Alzheimer's disease; stem cells; progenitors; neuroglia; intracellular signaling molecules; inhibitors.

1. Introduction

An important feature of the pathogenesis of neurodegenerative diseases is a disruption in the activity of cell renewal systems [1, 2]. Alzheimer's disease (AD) is the most common neurodegenerative disease. Moreover, in recent years there has been an explosive spread of AD and a decrease in the age of its manifestation [3-5].

According to modern concepts, the balance and efficiency of neurogenesis depend, first of all, on the activity of postnatal regenerative-competent cells (RCCs) of the central nervous system (CNS). Such cells include not only multipotent neural stem cells (NSC) [6]), but also lineage-restricted precursors, including neuronal committed progenitors (NCP) [7, 8]. At the

same time, the coordinated functioning of different populations of progenitors is important for neurogenesis [1, 7].

Therefore, it seems expedient to study the possibility of pharmacological stimulation and coordination of NSC and NCP functions for treating neurodegenerative diseases (including AD) [5, 9]. The development of such methods looks to be especially promising within the framework of the strategy of targeting intracellular signaling molecules in progenitor cells [10–14]. This approach involves a selective effect on the signaling pathways that regulate cell proliferation and differentiation. The selectivity of stimulation of tissue repair, in this case, is determined by the features of intracellular signaling in tissue-specific progenitor cells, as well as the specificity of expression of different types and isoforms (including protein products of alternative splicing) of signaling molecules [11, 15–17].

The etiology of AD is still unknown [18, 19]. However, the important role of β-amyloid () in its pathogenesis has been proven [20]. It is known that Aβ aggregates are one of the main causes of disruption of neuronal activity and the loss of the ability of the nervous tissue for balanced neurogenesis [21, 22]. However, many aspects of the regulation of the functions of progenitors under conditions of β-amyloid-induced neurodegeneration have not yet been studied. In particular, there is no detailed understanding of the role of some intracellular signaling in regulating cell functions in individual compartments of the nervous tissue cell renewal system. For example, the specificity of several individual molecules of MAPK- [9, 23], JAK/STAT- [2], and cAMP-mediated [17, 24] signaling pathways in the regulation of NSC and NCP functions in AD is not known. Studying these molecular mechanisms of the functioning of the nervous tissue RCCs under conditions of β-amyloid-induced neurodegeneration will make it possible to identify new promising drug targets for treating dementia of Alzheimer's type.

It may be very interesting to develop methods for targeted regulation of intracellular signal transduction of progenitors, considering the presence of a population of dormant stem cells in the CNS in the postnatal period [25]. Furthermore, there is convincing evidence of a significant role of somatic mutations (including transcriptional mutagenesis) in neurons in the development of AD [8, 26]. Based on this, it is logical to think that stimulation of dormant NSC that do not have such a genetic defect will lead to the formation of "healthy" mature nerve cells.

The work aimed to study the effect of inhibitors of MEK1, p38α/p38β, NF-κB, JAKs, STAT3, adenylate cyclase (AC), and PKA on the functions of NSC and NCP under conditions of modeling β-amyloid-induced neurodegeneration *in vitro*.

2. Materials and Methods

2.1. Chemicals and drugs.

The serum-free MACS Neuro Medium; anti-PSA-NCAM MicroBeads; (all manufactured by Miltenyi Biotec, Germany); Amyloid β-Protein Fragment 25-35; MEK1 inhibitor (PD98059); p38α/p38β inhibitor (SB202190); NF-κB inhibitor JSH-23 (J4455); JAKs inhibitor (Pan JAK Inhibitor «Ruxolitinib»); STAT3 inhibitor (STAT3 inhibitor XII, SPI); adenylate cyclase inhibitor (2′,5′-Dideoxyadenosine); PKA inhibitor (KT 5720); hydroxyurea (all manufactured by Calbiochem, Germany).

2.2. Animal.

Studies were carried out on 30 C57B1/6 mice at the age of 2-2.5 months. Before the beginning of experiments (during 10 days) and over the study period, animals were contained in a vivarium (air temperature 20–22°C, humidity 50-60 %) in plastic cages (10 mice) on a normal diet (solid diet pellets (LLC «Assortiment Firm», Sergiev Posad city, Russia), water ad libitum. To exclude seasonal variations from the parameters studied, all experiments were conducted in winter. The dark/light cycle was 12/12 hours. After the experiments, the mice were sacrificed with a CO_2 camera. The experiments were approved by the Ethics Committee of Goldberg Research Institute of Pharmacology and Regenerative Medicine (protocol GRIPh & RM-2022-01/12).

2.3. Experimental design.

In vitro experiments were used to study the direct effect of the inhibitors of MEK1 (100 μM), p38α/p38β (10 μM), NF-κB (30 μM), JAKs (200 nM), STAT3 (10 μM), AC (30 μM) and PKA (10 μM) on the realization of the growth potential of NSC and NCP [1, 7] under the conditions of modeling β-amyloid-induced neurodegeneration *in vitro* was studied. The appropriate cell cultures with Aβ without signaling molecule inhibitors served as controls.

2.4. Modeling β-amyloid-induced neurodegeneration.

The Aβ fragment 25-35 was used in the experiment. To obtain protein aggregates, it was incubated at a concentration of 1 mM for 7 days at 37°C, 5% CO_2, and 100% air humidity. The aggregated Aβ was added to the culture medium *in vitro* to a final concentration of 20 μM [27].

2.5. NSC and NCP study.

The nervous tissue to produce cellular material was extracted from the subventricular area of the cerebral hemispheres (SVZ). NSC was prepared from unfractionated SVZ cells. To study NCP using an immunomagnetic separator "MIniMACS Cell Separator" (Miltenyi Biotec, Germany). It isolated PSA-NCAM$^+$ (CD56$^+$) cells (by positive selection) [1]. The cells at a concentration of 10^5 / ml were incubated in MACS Neuro Medium for 5 days in a CO_2 incubator (under standard conditions). After incubation, the content of clonogenic cells, their mitotic activity, and the specialization intensity was calculated. The yield in the respective cultures of colony-forming units (CFU, neurospheres containing more than 100 cells) determined the number of NSC and NCP. The proliferative activity of CFU was assessed by the method of cell suicide using an agent specifically inhibiting DNA synthesis enzymes in the S-phase of the cell cycle (hydroxyurea (at a concentration of 1 μM)). The specialization (differentiation/maturation) intensity of progenitors was determined by calculating the ratio of the corresponding cluster-forming units (ClFU, neurospheres of 30 - 100 cells) to CFU (specialization index) [1, 8].

2.6. Statistical analysis.

The data were analyzed with one-way ANOVA followed by Dunnett's test, Wilcoxon's test for dependent samples, and Mann-Whitney test for independent samples. The data are expressed as arithmetic means. The significance level was $p < 0.05$ [28].

3. Results and Discussion

3.1. Effect of Aβ on NSC and NCP functions.

The addition of aggregated Aβ to the culture medium caused an ambiguous reaction on the part of the colony-forming capacity of different progenitors. In the culture of unfractionated cells from SVZ, a decrease in the number of CFU_{NSC} was observed (up to 76.3% of the similar value in the cell culture without Aβ) (Figure 1, A). At the same time, Aβ stimulated the clonogenic activity of $CD56^+$ cells. The number of CFU_{NCP} increased to 346.7% of the initial level (Figure 2, A). These changes reflected the corresponding changes in the mitotic activity of both types of progenitors. There was a decrease in the intensity of NSC proliferation (up to 71.5% of the corresponding parameter in the medium without Aβ) and an increase in this indicator in NCP (up to 206.1% of the initial level) (Figure 1, B; 2, B). Against this background, the index of specialization of multipotent progenitors increased (up to 183.1% of the initial level), while in committed precursors, on the contrary, it significantly decreased and reached 55.6% of the same indicator in control (medium without Aβ) (Figure 1, C; 2, C).

The results of the experiments were in full agreement with our earlier data on the opposite reaction of NSC and NCP to Aβ [29]. This uncoupling of the activity of nervous tissue progenitors of different types is one of the main causes of neurogenesis disorders in AD.

3.2. Targeting of signaling pathways in NSC and NCP.

3.2.1. MAPK signaling pathway.

The MEK1 and p38α/p38β inactivation caused in many respects the same type of changes in the functioning of NSC both under conditions of their optimal vital activity and in modeling neurodegeneration. In both cases, there was an increase in the level of colony formation in the culture of unfractionated cells from SVZ (Figure 1, A). These changes were more pronounced when exposed to a neurotoxic agent. The number of CFU_{NSC}, in this case, increased to 140.3% of the control value (while in the culture medium without Aβ, the increase in the parameter reached only 119.4% of the initial level). The change in the NSC colony-forming capacity was the result of an increase in their proliferative activity: up to 121.6% and 237.3% of the corresponding control levels (in the cell culture without Aβ and with the addition of Aβ) (Figure 1, B).

Also, regardless of the cultivation conditions, the NSC intensity differentiation changed in the same way during p38α/p38β blockade. In both cases, a decrease in this indicator was observed. The MEK1 inhibitor affected this parameter only under conditions of modeling Aβ-induced neurodegeneration. The value of the differentiation index, in this case, was 74.8% of the initial level (Figure 1, C).

The committed progenitor cells responded significantly less to MAPK inhibitors. The p38α/p38β blockade influenced the functioning of clonogenic $CD56^+$ cells only under conditions of their optimal vital activity (medium without Aβ). In this experimental group, an increase in the number of CFU_{NCP} and their proliferative activity was observed (up to 163.7% and 124.6% of the initial levels, respectively). Inactivation of p38 in the culture of $CD56^+$ cells with Aβ did not cause any changes. At the same time, the MEK1 inhibitor had no effect on NCP (Figure 2).

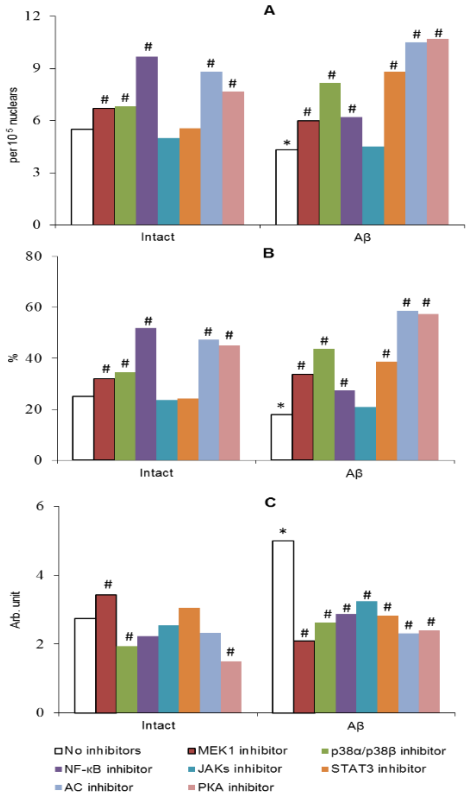

Figure 1. (A) Amount of CFU_{NSC}; **(B)** NSC proliferative activity; **(C)** NSC differentiation index**.** Here and in Figure 2: cell culture without βA (intact); with βA. * - differences with intact; # - differences with the group without inhibitors at $p < 0.05$.

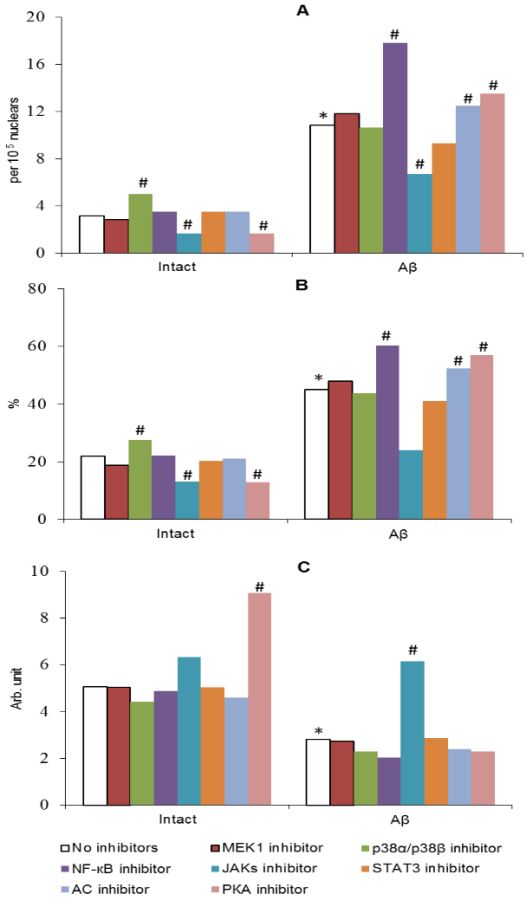

Figure 2. (A) Amount of CFU_{NCP}; **(B)** NCP proliferative activity; **(C)** NCP differentiation index.

Thus, inhibitors of MEK1 and p38α/p38β, on the one hand, abolished the negative effect of Aβ on the NSC functioning and, on the other hand, did not affect the compensatory activation of NPCs proliferation induced by neurotoxic agents.

3.2.2. NF-κB-signaling pathway.

The experiment revealed an important role of NF-κB signaling in the regulation of NSC functions. The NF-κB inhibitor, both in the amyloid-free medium and in the simulation of Aβ-induced neurodegeneration, led to a significant increase in the number of CFU_{NSC} and their proliferative activity (up to 178.3% and 208.1% of the corresponding control values under conditions of optimal vital activity; and 144.6% and 147.5% of the initial levels when exposed to Aβ, respectively) (Figure 1, A, B). Inactivation of NF-κB against the background of exposure to a neurotoxic agent also caused a decrease in the NSC intensity of differentiation (up to 70.4% of the background value) (Figure 1, C).

Other patterns were revealed in the implementation of the growth potential of NCP under the influence of an NF-κB inhibitor. In the Aβ-free medium, NF-κB blockade did not change the functioning of committed progenitors (Figure 2). However, modeling of neurodegeneration was accompanied by a pronounced increase in the number of CFU_{NCP} (up to 167.2% of the initial level) and their mitotic activity (up to 144.9% of the initial level) in response to the introduction of amyloid into the $CD56^+$ cells culture. At the same time, the intensity of NCP maturation practically did not change (Figure 2, C).

Thus, the NF-κB blockade in the NSC and NCP significantly synchronized their activities.

3.2.3. JAKs/STAT-signaling pathway.

The study of the JAK/STAT pathway made it possible to reveal a number of features of their participation in the realization of the growth potential of various precursors of nervous tissue. Adding the JAKs and STAT3 inhibitors to the medium without Aβ did not affect the functioning of NSC *in vitro* (Figure 1). In contrast, under the conditions of modeling neurodegeneration, blockade of STAT3 led to an increase in the number of CFU_{NSC} in the culture of unfractionated cells of the nervous tissue and their proliferative activity (up to 203.2% and 215.7% of similar values in the medium with Aβ without signaling molecule inhibitors). At the same time, there was a pronounced decrease in the intensity of NSC differentiation to the level of this indicator in cell culture without Aβ. Similar changes in the specialization of NSC were recorded during the blockade of JAKs under the influence of a neurotoxic agent. However, JAKs inactivation did not cause other changes in the functioning of the NSC.

Dissimilar committed progenitors reacted to the blockade of these signaling molecules. Under conditions of optimal vital activity, changes in NCP functions were observed only under the influence of the JAKs inhibitor. It reduced the colony-forming capacity of $CD56^+$ cells by inhibiting their proliferation (up to 52.7% and 59.6% of the control values, respectively) (Figure 2, A, B). When modeling neurodegeneration, these changes in the functioning of the NCP under the influence of the JAKs blocker persisted. However, an additional increase in their specialization intensity was noted (up to the initial level - a medium without Aβ and inhibitors of signaling molecules) (Figure 2). The STAT3 blockade, as well as in the Aβ-free medium, did not change the implementation of the functions of NCP.

Thus, the inactivation of STAT3 under conditions of modeling Aβ-induced neurodegeneration led to the leveling of the negative effect of the neurotoxic agent on the functions of NSC and stimulation of their proliferation. However, unlike the JAKs blocker, the STAT3 inhibitor did not suppress the compensatory progression of the cell cycle of NCP caused by Aβ.

3.2.4. cAMP-signaling pathway.

The important role of cAMP and PKA in regulating various types of progenitors was revealed, regardless of their cultivation conditions *in vitro*. The blockade of AC and PKA in both cases (medium without Aβ and with Aβ) was accompanied by a pronounced stimulation of colony formation of unfractionated cells of the nervous tissue. Moreover, the highest increase in the number of CFU_{NSC} was observed when modeling Aβ-induced neurodegeneration (up to 242.5% and 243.1% of the background in AC and PKA blockade, respectively) (Figure 1, A). This was associated with significant activation of NSC proliferation (up to 32.6% and 31.9% of the corresponding control level of the indicator in the inactivation of AC and PKA). (Figure 1, B). Conversely, violation of cAMP-mediated signal transduction led to a decrease in the intensity of NSC differentiation almost to the level of this indicator in the medium without Aβ and signaling molecule inhibitors (Figure 1, C).

The role of the cAMP-pathway in committed neuronal progenitors differed from that in NSC. The blockade of AC did not affect the functioning of NCP both under conditions of optimal vital activity *in vitro* and when cultivated in a medium with Aβ. But NCP reacted to the PKA inhibitor. Moreover, in the Aβ-free medium, the colony formation levels and the NCP's proliferative activity decreased under the influence of this pharmacological agent (Figure 2, A, B). At the same time, their maturation accelerated (up to 179.1% of the initial level) (Figure 2, C). Under conditions of simulation of Aβ-induced neurodegeneration, PKA inactivation caused an increase in the mitotic activity of committed precursors (up to 126.7% of the control level).

Thus, the blockade of cAMP synthesis and selective inactivation of PKA significantly stimulated NSC proliferation. Moreover, under conditions of neurotoxic exposure, these changes were most pronounced. However, with regard to committed neuronal progenitors, only the PKA inhibitor had such an effect in modeling neurodegeneration.

In general, the experimental results confirm our earlier data on the formation of a fundamentally new pattern of intracellular signaling in progenitors under the influence of a fragment of neurotoxic β-amyloid [29]. The experiments revealed the desynchronization of the activities of the NSC and the NCP. Opposite changes in their proliferation and differentiation status were found under conditions of βA-induced neurodegeneration. In particular, inhibition of NSC proliferation against the background of progression of the NCP cell cycle was revealed. At the same time, the change in the intensity of specialization of progenitors in the first case increased, while in the second, on the contrary, it decreased (Figure 3). Thus, the experiments demonstrate the extraordinary phenomenon of discoordination of the activities of NSC and NCP against the background of dissociation of their proliferation and differentiation processes. These disturbances in the system of cellular renewal of the nervous tissue system underlie inefficient neurogenesis in AD [30, 31]. Moreover, the intensification of the rate of NSC differentiation should also be considered a pathogenic factor since it is known that a high rate of precursor specialization often causes aberrant cell development [32, 33]. Therefore, the main

efforts in developing methods for stimulating CNS repair in AD should be directed at finding ways to coordinate the activity of regenerative-competent cells of various types [1, 8].

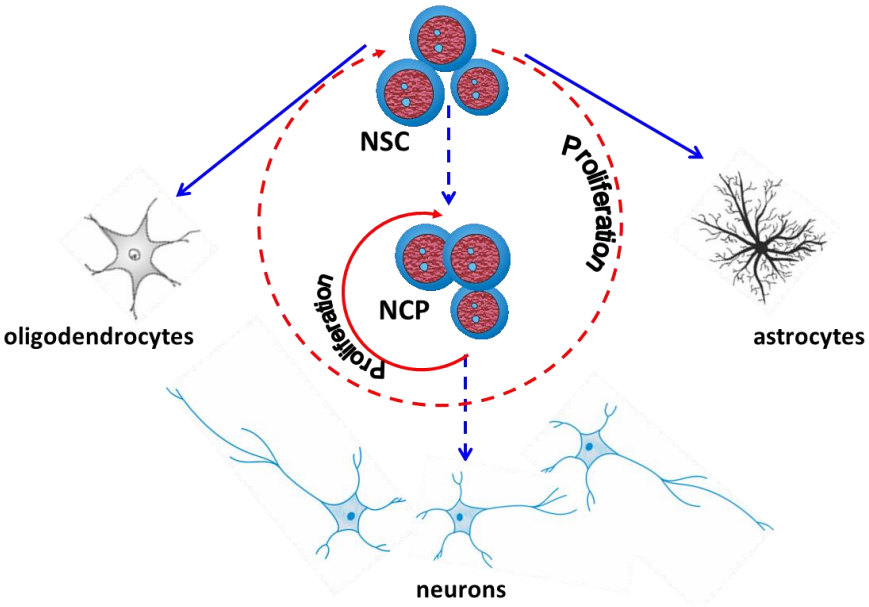

Figure 3. Discoordination of the activities of NSC and NCP against the background of disunity of their proliferation and differentiation in AD. Solid lines - stimulation; dotted lines - braking.

The experiments have shown that, under conditions of βA-induced neurodegeneration, new patterns of intracellular signal transduction in NSC and NPCs arise, which are responsible for the realization of their growth potential. In contrast, a unique possibility of coordinating the pro-regenerative properties of different types of progenitors was revealed due to the targeted regulation of signal transduction in them [15–17]. The inhibitors of MEK1, p38α/p38β, NF-κB, STAT3, and PKA were found to be the most effective correctors of NSC and NCP dysfunctions. The selective blockade of each of these signaling molecules was accompanied by the leveling of the negative effect of βA on multipotent progenitors and the preservation (and in the case of inactivation of NF-κB and PKA, even by additional stimulation) of a pronounced compensatory reaction of committed neuronal precursors (Figure 4).

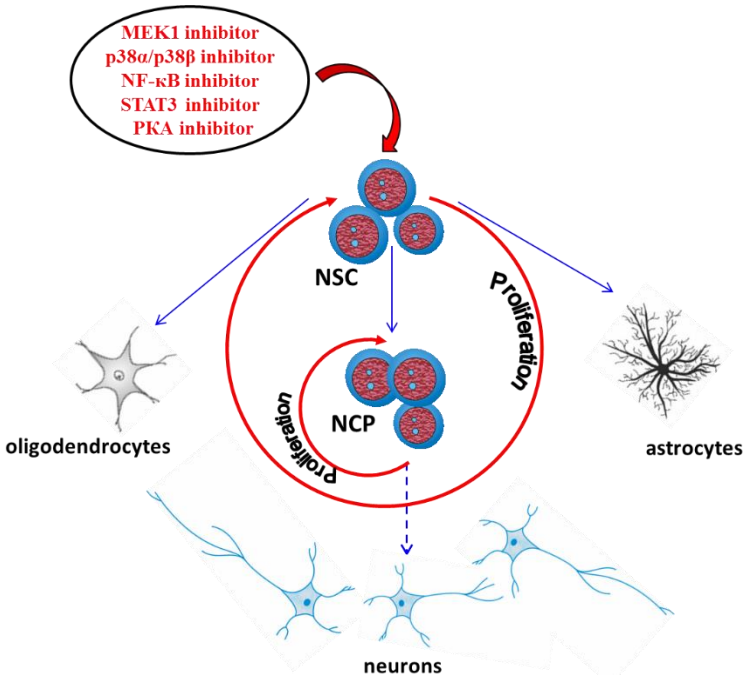

Figure 4. The activity of NSC and NCP in AD under the influence of signaling molecule inhibitors. Thick lines - stimulation; thin lines - no effect

At the same time, the important role of neuroglia in CNS recovery should be taken into account [34]. Both macroglial and microglial cells are involved in the repair of nervous tissue through intercellular communication with progenitors and the secretion of cytokines (stimulators and inhibitors of neurogenesis) [2, 35]. In this regard, it is reasonable to study the effect of these inhibitors of intracellular signaling molecules on the functioning of different populations of glial cells in the simulation of βA-induced neurodegeneration. Such experiments will allow evaluation of the possibility of achieving conjugation between the pro-regenerative functions of progenitors and the activity of neuroglial cells using targeted signal transduction regulators.

4. Conclusions

The findings indicate the prospect of drug discovery for the treatment of AD based on inhibitors of MEK1, p38α/p38β, NF-κB, STAT3 и PKA This expands the horizon for the use of modifiers of the activity of intracellular signaling molecules and opens up new perspectives for implementing a strategy for targeted regulation of intracellular pathways.

Funding

The study was carried out at the expense of a grant from the Russian Science Foundation No. 22-25-00069, https://rscf.ru/project/22-25-00069/.

Acknowledgments

We thank the Director of the Goldberg Research Institute of Pharmacology and Regenerative Medicine, V.V. Zhdanov, for providing research infrastructure for the work.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Zyuz'kov, G.N.; Miroshnichenko, L.A.; Polyakova, T.Yu.; Simanina, E.V.; Stavrova, L.A. Targeting cAMP-pathway in Regeneration-Competent Cells of Nervous Tissue: Potential to Create a Novel Drug for Treatment of Ethanol-Induced Neurodegeneration. *Central Nervous System Agents in Medicinal Chemistry* **2021**, *21*, 172-180, https://doi.org/10.2174/1871524921666210907102847.
2. Zyuz'kov, G.N.; Zhdanov, V.V.; Miroshnichenko, L.A.; Polyakova, T.Yu.; Simanina, E.V.; Danilets, M.G.; Minakova, M.Yu.; Churin, A.A.; Agafonov, V.I. The Role of JAK and STAT3 in Regulation of Secretory Function of Neuroglial Cells of Different Types in Ethanol-Induced Neurodegenerationt. *Bulletin of experimental biology and medicine* **2022**, *172*, 686-690, https://doi.org/10.1007/s10517-022-05457-8.
3. Drummond, E.; Pires, G.; MacMurray, C.; Askenazi, M.; Nayak S.; Bourdon, M.; Safar, J.; Ueberheide, B.; Wisniewski, T. Phosphorylated tau interactome in the human Alzheimer's disease brain. *Brain* **2020**, *143*, 2803-2817, https://doi.org/10.1093/brain/awaa223.
4. Han, F.; Bi, J.; Qiao, L.; Arancio, O. Stem Cell Therapy for Alzheimer's Disease. *Advances in experimental medicine and biology* **2020**, *1266*, 39-55. https://doi.org/10.1007/978-981-15-4370-8_4.
5. Vaz, M.; Silvestre, S. Alzheimer's disease: Recent treatment strategies. *European journal of pharmacology* **2020**, *887*, 173554. https://doi.org/10.1016/j.ejphar.2020.173554.
6. Zhang, X.; Mei, Y.; He, Y.; Wang, D.; Wang, J.; Wei, X.; Yang, E.; Zhou, D.; Shen, H.; Peng, G.; Shu, Q.; Li, X.; Luo, B.; Zhou, Y.; Sun, B. Ablating Adult Neural Stem Cells Improves Synaptic and Cognitive Functions in Alzheimer Models. *Stem Cell Reports* **2021**, *16*, 89-105, https://doi.org/10.1016/j.stemcr.2020.12.003.

7. Zyuz'kov, G.N.; Miroshnichenko, L.A.; Polyakova, T.Yu.; Stavrova, L.A.; Simanina, E.V.; Zhdanov, V.V.; Chaikovskii, A.V. Peculiarities of the Involvement of MAPKS ERK1/2 and p38 in the Implementation of the Functions of Neural Stem Cells and Neuronal Committed Precursors in Ethanol-Induced Neurodegeneration. *Bulletin of Experimental Biology and Medicine* **2020**, *169*, 609-613, https://doi.org/10.1007/s10517-020-04938-y.
8. Zyuz'kov, G.N.; Miroshnichenko, L.A.; Chayikovskyi, A.V.; Kotlovskaya, L.Yu. NF-κB: a target for synchronizing the functioning nervous tissue progenitors of different types in Alzheimer's disease. *Current Molecular Pharmacology* **2022**, *15*, https://dx.doi.org/10.2174/1874467215666220601144727.
9. Zyuz'kov, G.N.; Miroshnichenko, L.A.; Polyakova, T.Yu.; Zhdanov, V.V.; Simanina, E.V.; Stavrova, L.A.; Churin, A.A.; Fomina, T.I. Role of MAPK ERK1/2 and p38 in the Regulation of Secretory Functions of Different Populations of Neuroglia in Ethanol-Induced Neurodegeneration. *Bulletin of Experimental Biology and Medicine* **2021**, *171*, 699-703, https://doi.org/10.1007/s10517-021-05298-x.
10. Jha, N.K.; Jha, S.K.; Kar, R.; Nand, P.; Swati, K.; Goswami, V.K. Nuclear factor-kappa beta as a therapeutic target for Alzheimer's disease. *Journal of neurochemistry* **2019**, *150*, 113-137, https://doi.org/10.1111/jnc.14687.
11. Chen, W.T.; Lin, G.B.; Kuo, Y.Y.; Hsieh, C.H.; Lu, C.H.; Sun, Y.K.; Chao, C.Y. Effect of high-frequency low-intensity pulsed electric field on protecting SH-SY5Y cells against hydrogen peroxide and beta-amyloid-induced cell injury via ERK pathway. *PLoS One* **2021**, *16*, e0250491, https://doi.org/10.1371/journal.pone.0250491.
12. Bryushinina, O.S.; Zyuz'kova, Yu.G.; Yanovskaya, E.A.; Abdrashitova, N.Y.; Frelikh, G.A.; Lakeev, A.P.; Tsuran, D.V.; Udut, V.V.; Zyuz'kov, G.N. Role of JNK in the Regulation of Xenobiotic Metabolizing Function of Hepatocytes. *Bulletin of Experimental Biology and Medicine* **2021**, *172*, 146-150, https://doi.org/10.1007/s10517-021-05352-8.
13. Ohgomori, T.; Jinno, S. Signal Transducer and Activator of Transcription 3 Activation in Hippocampal Neural Stem Cells and Cognitive Deficits in Mice Following Short-term Cuprizone Exposure. *Neuroscience* **2021**, *472*, 90-102, https://doi.org/10.1016/j.neuroscience.2021.07.031.
14. Wang, Z.; Chen, Y.4 Li, X.; Sultana, P.; Yin, M.; Wang Z. Amyloid-beta(1-42) dynamically regulates the migration of neural stem/progenitor cells via MAPK-ERK pathway. *Chemico-biological interactions* **2019**, *298*, 96-103, https://doi.org/10.1016/j.cbi.2018.11.001.
15. Zyuz'kov G.N. Targeted Regulation of Intracellular Signal Transduction in Regeneration-Competent Cells: A new Direction for Therapy in Regenerative Medicine. *Biointerface Research in Applied Chemistry* **2021**, *11*, 12238-12251, https://doi.org/10.33263/BRIAC114.1223812251.
16. Zyuz'kov, G.N.; Miroshnichenko, L.A.; Simanina, E.V.; Stavrova, L.A.; Polyakova, T.Yu. Intracellular signaling molecules of nerve tissue progenitors as pharmacological targets for treatment of ethanol-induced neurodegeneration. *Journal of basic and clinical physiology and pharmacology* **2022**, *33*, 305-315, https://doi.org/10.1515/JBCPP-2020-0317.
17. Zyuz'kov, G.N.; Miroshnichenko, L.A.; Polyakova, T.Yu.; Stavrova, L.A. ; Simanina, E.V. Wound healing properties of the protein kinase A inhibitor and the mechanisms of their development. *Bangladesh Journal of Pharmacology* **2021**, *16*, 34-41, https://doi.org/10.3329/bjp.v16i2.50575.
18. Scheltens, P.; De Strooper, B.; Kivipelto, M.; Holstege, H.; Chételat, G.; Teunissen, C.E.; Cummings, J.; van der Flier, W.M. Alzheimer's disease. *Lancet* **2021**, *397*, 1577-1590, https://doi.org/10.1016/S0140-6736(20)32205-4.
19. Serrano-Pozo, A.; Das, S.; Hyman, B.T. APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. *The Lancet. Neurology* **2021**, *20*, 68-80, https://doi.org/10.1016/S1474-4422(20)30412-9.
20. Ashrafian, H.; Zadeh, E.H.; Khan, R.H. Review on Alzheimer's disease: Inhibition of amyloid beta and tau tangle formation. *International journal of biological macromolecules* **2021**, *167*, 382-394, https://doi.org/10.1016/j.ijbiomac.2020.11.192.
21. Gallardo, G.; Holtzman, D.M. Amyloid-beta and Tau at the Crossroads of Alzheimer's Disease. *Advances in experimental medicine and biology* **2019**, *1184*, 187-203, https://doi.org/10.1007/978-981-32-9358-8_16.
22. Luu, L.; Ciccotosto, G.D.; Cappai, R. The Alzheimer's Disease Amyloid Precursor Protein and its Neuritogenic Actions. *Current Alzheimer research* **2021**, *18*, 772-786, https://doi.org/10.2174/1567205018666211208141017.
23. Cheng, C.Y.; Huang, H.C.; Kao, S.T.; Lee, Y.C. Angelica sinensis extract promotes neuronal survival by enhancing p38 MAPK-mediated hippocampal neurogenesis and dendritic growth in the chronic phase of

transient global cerebral ischemia in rats. *Journal of ethnopharmacology* **2021**, *278*, 114301, https://doi.org/10.1016/j.jep.2021.114301.
24. Huang, P.; Wei, S.; Luo, M.; Tang, Z.; Lin, Q.; Wang, X.; Luo, M.; He, Y.; Wang, C.; Wei, D.; Xia, C.; Xu, J. MiR-139-5p has an antidepressant-like effect by targeting phosphodiesterase 4D to activate the cAMP/PKA/CREB signaling pathway. *Annals of translational medicine* **2021**, *9*, 1594, https://doi.org/10.21037/atm-21-5149.
25. Kim, H.; Cho, B.; Park, H.; Kim, J.; Kim, S.; Shin, J.; Lengner, C.J.; Won, K.J.; Kim, J. Dormant state of quiescent neural stem cells links Shank3 mutation to autism development. *Molecular psychiatry* **2022**, *27*, 2751-2765, https://doi.org/10.1038/s41380-022-01563-1.
26. Kaeser, G.; Chun, J. Brain cell somatic gene recombination and its phylogenetic foundations. *The Journal of biological chemistry* **2020**, *295*, 12786-12795, https://doi.org/10.1074/jbc.REV120.009192.
27. Coronel, R.; Lachgar, M.; Bernabeu-Zornoza, A.; Palmer, C.; Domínguez-Alvaro, M.; Revilla, A.; Ocaña, I.; Fernández, A.; Martínez-Serrano, A.; Cano, E.; Liste I. Neuronal and Glial Differentiation of Human Neural Stem Cells Is Regulated by Amyloid Precursor Protein (APP) Levels. *Molecular neurobiology* **2019**, *56*, 1248-1261, https://doi.org/10.1007/s12035-018-1167-9.
28. Curtis, M.J.; Bond, R.A.; Spina, D.; Ahluwalia, A.; Alexander, S.P.; Giembycz, M.A. Experimental design and analysis and theirreporting: new guidance for publication in BJP. *British journal of pharmacology* **2015**, *172*, 3461–3471, https://doi.org/10.1111/bph.12856.
29. Zyuz'kov, G.N.; Miroshnichenko, L.A.; Chayikovskyi, A.V.; Kotlovskaya, L.Yu. 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. *Bulletin of Experimental Biology and Medicine* **2022**, *173*, 424-428, https://doi.org/10.1007/s10517-022-05561-9.
30. Liu, H.; Zhang, H.; Ma, Y. Molecular mechanisms of altered adult hippocampal neurogenesis in Alzheimer's disease. *Mechanisms of ageing and development* **2021**, *195*, 111452, https://doi.org/10.1016/j.mad.2021.111452.
31. Li Puma, D.D.; Piacentini, R.; Grassi, C. Does Impairment of Adult Neurogenesis Contribute to Pathophysiology of Alzheimer's Disease? A Still Open Question. *Frontiers in molecular neuroscience* **2021**, *13*, 578211, https://doi.org/10.3389/fnmol.2020.578211.
32. Fliedner, A.; Gregor, A.; Ferrazzi, F.; Ekici, A.B.; Sticht, H.; Zweier, C. Loss of PHF6 leads to aberrant development of human neuron-like cells. *Scientific reports* **2020**, *10*, 19030, https://doi.org/10.1038/s41598-020-75999-2.
33. Wilkes, M.C.; Shibuya, A.; Sakamoto, K.M. Signaling Pathways That Regulate Normal and Aberrant Red Blood Cell Development. *Genes (Basel)* **2021**, *12*, 1646, https://doi.org/10.3390/genes12101646.
34. Araki, T.; Ikegaya, Y.; Koyama, R. The effects of microglia- and astrocyte-derived factors on neurogenesis in health and disease. *The European journal of neuroscience* **2021**, *54*, 5880-5901, https://doi.org/10.1111/ejn.14969.
35. Ozben, T.; Ozben S. Neuro-inflammation and anti-inflammatory treatment options for Alzheimer's disease. *Clinical biochemistry* **2019**, *72*, 87-89. https://doi.org/10.1016/j.clinbiochem.2019.04.001.