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Receptor-mediated Oxidative Stress in Murine Cerebellar Neurons is Accompanied by Phosphorylation of MAP (ERK 1/2) Kinase

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Abstract: A primary culture of murine cerebellar neurons was used to induce oxidative stress resulting in the accumulation of reactive oxygen species (ROS) and activation of ERK 1/2 kinase. Short-term incubation (15 min) of cerebellar neurons with homocysteine (HC) or N-methyl-D-aspartate (NMDA) induced partial ERK 1/2 phosphorylation thus providing the activation of the enzyme. Inhibitors of NMDA receptors, MK-801 or D-AP5, both prevented the activation of cells by HC or NMDA. Another receptor-dependent means of oxidative stress stimulation is exposure of cells to the cardiac glycoside ouabain, a specific inhibitor of Na/K-ATPase. Ouabain induces ROS accumulation and substantial ERK1/2 activation in neuronal cells at concentrations as low as 1 nM – 1 M, which corresponds to participation of Na/K-ATPase in intracellular signalling. Neuropeptide carnosine added to the cells 2 hours before oxidative stress prevented both ROS accumulation and ERK1/2 activation. As ERK1/2 kinase plays a key role in gene expression responsible for either cell adaptation or cell death, the model used gives a useful tool to characterize the effect of natural and synthetic anti-cancer drugs on cellular life. The data presented show that carnosine is a natural modulator of oxidative stress in neuronal cells, providing regulation of ERK1/2 activity *via* buffering intracellular ROS levels.

Keywords: Carnosine, ERK 1/2, homocysteine, Na/K-ATPase, neurons, NMDA, ouabain, oxidative stress.

1. INTRODUCTION

During its lifespan the cell is affected by various exogenous factors (change of temperature, osmotic pressure, action of chemical substances, growth factors, cytokines, etc.) activating multiple response reactions (such as division, differentiation and apoptosis). The mechanism of activation of these reactions often boils down to activation of the same mitogen-activated protein kinase (MAPK) cascade. The MAPK family is a group of proteins participating in signal transmission from exogenous factors into the cell, thus playing an important role in regulation of cell proliferation, differentiation, and adaptation to environmental conditions [1].

ERK1/2 (extracellular-signal-regulated protein kinase, isoforms 1 and 2) is one of the most studied members of the MAPK family. It is an obligatory participant of normal development and function of the cerebral neurons. ERK1/2 signaling cascade is generally activated in response to growth factor, resulting in cell proliferation and differentiation. More and more data have been obtained recently showing that ERK1/2 is also activated under oxidative stress conditions inducing cell death processes [2, 3]. An increased level of phosphorylated ERK1/2 along with an increased concentration of reactive oxygen species (ROS) is observed in cerebral nervous tissue cells in Alzheimer's disease patients and in the case of parkinsonism and some other neurodegenerative diseases [4, 5].

2. FACTORS OF ERK 1/2 ACTIVATION IN NEU-RONS

One of the factors involving ERK 1/2 kinase in the signaling cascade is ionotropic glutamate receptors activated by *N*-methyl-D-aspartate (NMDA). NMDA receptors are obligatorily participants in the processes of synaptic integrity and plasticity, as well as in learning and memory [6-9]. NMDA receptor activation results in opening of its ion channel through which calcium ions enter the cell. One of the consequences of NMDA receptor activation is the increase in ROS level [10, 11], which may result in cell death. ROS accumulation is a Ca-dependent process because the use of calcium chelators prevents ROS increase and cell death [12]. Both calcium ions (Ca²⁺) and ROS may act as second messengers in the cell triggering cell-signaling cascades, in particular ERK1/2 cascade [13, 14].

A structural analog of glutamate — homocysteine (HC) — has a similar effect on NMDA receptors. It is a natural metabolite, a metabolic product of methionine, and is normally contained in the body (blood plasma, cerebrospinal fluid) in rather small quantities (10–12 μ M). An increase in HC concentration is characteristic of many neurodegenerative diseases, such as Parkinson's disease, schizophrenia, Alzheimer's disease, and alcohol dependence [15]. HC was shown to activate NMDA receptors, resulting in an increase in the intracellular level of Ca²⁺ and ROS and inducing apoptotic cell death [16].

To inactivate NMDA receptors, two specific inhibitors were used: D-AP5, which competes with high affinity with

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ligands for binding sites at the receptor molecule, and MK-801, which prevents the opening of a receptor-dependent ion channel. Both compounds were found to decrease the HCpromoting effect both on ROS production and cytoplasmic calcium levels. Here, inhibitors of metabotropic glutamate receptors have almost no effect on HC. Use of the antioxidant *N*-acetylcysteine (NAC) or the intracellular calcium chelator BAPTA also prevented ROS accumulation and the increase in the level of ionized calcium in the cell and likewise decreased cell death [17, 18]. Thus, similarly to NMDA, HC is able to activate NMDA receptors and imitate the excitotoxic effect of this mediator.

Another means of inducing oxidative stress in neurons is the inhibition of Na/K-ATPase by cardiotonic steroids. Na/K-ATPase is a key enzyme of external membranes, providing formation and maintaining of monovalent cation gradient on the cell membrane [19]. Short-term incubation of Na/K-ATPase by one of cardiotonic steroids, ouabain, resulted in an increase in intracellular Ca²⁺ concentration, mitochondrial membrane depolarization, and an increase in intracellular ROS levels [20-22]. Later on, triggering of Ca-dependent signaling cascades, ERK 1/2 kinase activation, and early response gene expression take place [23-25].

New data characterizing the functional interaction of NMDA receptors and Na/K-ATPase in neurons has appeared recently. For example, ouabain-induced changes in cells can be prevented by the above-mentioned inhibitors of NMDA receptors. In cultured rat cerebellar neurons, agonists of NMDA receptors (NMDA and HC) were shown to suppress the activity of Na/K-ATPase [26, 27]. This effect can probably be explained by accumulation of free radicals as a result of cell receptor activation, because it was weakened in the presence of ionotropic glutamate receptor antagonists. It was also reported that the HC effect develops more slowly than NMDA effect [28].

3. ERK1/2 ACTIVATION DYNAMICS WITH THE PARTICIPATION OF NMDA RECEPTORS

To study time dependency of ERK1/2 activation on NMDA receptor activation, rat cerebellar granule cells were



incubated with NMDA receptor ligands, NMDA and HC (incubation conditions are described in figure legends).

As shown in Fig. (1), the ERK1/2 activation time profile in the presence of the two ligands used is different: NMDA induced rapid ERK1/2 activation after only 2.5 min, and then a dramatic decline in the protein kinase activity to the control level was observed. HC induced a more sloped increase in phospho-ERK1/2 level and more continuous signal retention; the maximum value was observed 10 min after incubation. A complete reduction of ERK1/2 activity to the control level in the presence of HC was not observed even after 30 min of incubation.

To confirm the role of NMDA receptors in ERK1/2 activation in the presence of HC, specific antagonists of NMDA receptors, D-AP5 and MK-801, were used. The cells were preincubated with these antagonists and then exposed to HC (Fig. 2). The figure shows that preincubation with D-AP5 almost completely prevented ERK1/2 activation under the action of NMDA, and MK-801 exerted a similar effect. On the basis of the known mechanism of action of these antagonists, we suggest that NMDA receptor activation by HC induces ERK1/2 kinase phosphorylation associated with the entering of Ca²⁺ into the cell *via* the NMDA receptor ion channel.

To confirm this, we eliminated calcium ions from the external solution. Then the neurons were exposed to HC or NMDA, and the level of the active form of ERK1/2 was evaluated by flow cytometry. The data obtained show (Fig. 3) that in the absence of calcium ions ERK1/2 is not activated. Thus, extracellular calcium is an obligatory participant in ERK1/2 activation under oxidative stress conditions when NMDA receptors are activated.

4. INHIBITION OF Na/K-ATPase RESULTS IN ERK1/2 KINASE ACTIVATION

The dependency of the activity of neuronal Na/K-ATPase on increasing concentrations of ouabain has a biphasic curve shape, which corresponds to two types of the enzyme α -subunit isoforms with high and low sensitivity to the inhibitor [29]. As mentioned above, incubation of neurons with ouabain induces ROS production in the cell. It is reasonable





Fig. (1). Time dependency of ERK1/2 activation in cerebellar neurons under the action of 500 μ M NMDA (A) or HC (B). Quantification of phosphorylated form of the enzyme was done by the immunoblotting method, using antibodies against the phosphorylated form of ERK1/2. Calculated values were normalized to the control values, which were considered to be 100%.

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to suppose that under these conditions ERK1/2 kinase is also activated.

To study the time dependency of ouabain's effect on ERK1/2 activity in cerebellar granule cells, this inhibitor was taken in two different concentrations, inactivating mostly the population of ouabain-sensitive enzyme molecules (1 nM) and also affecting ouabain-resistant molecules (1 μ M) – see Fig. (4).

Fig. (4) shows that ERK1/2 activation was already observed 5 min after adding ouabain. The character of the activation depended on its concentration: in the presence of a low concentration of ouabain (1 nM), ERK1/2 activity reached its maximum in 10 min and then decreased. In the presence of a high concentration of ouabain (1 μ M), ERK1/2 activation was more prolonged. Thus, involving Na/K-ATPase isoforms with different ouabain sensitivity induces two types of ERK1/2 activation similar to that observed when NMDA receptors were activated by NMDA or HC (Fig. 1).

5. EFFECT OF CARNOSINE ON ERK1/2 ACTIVA-TION UNDER THE ACTION OF NMDA AND HC

The specific class of substances able to protect the cell against free-radical action is commonly referred to as antioxidants. One of these substances is the natural histidinecontaining dipeptide carnosine, a derivative of β -alanine and L-histidine. Carnosine is a neuropeptide that accumulates in high concentrations (5–20 mM) in the excitable tissues of vertebrates (skeletal muscles, myocardium, cerebrum). It is able to perform different functions because it is a natural buffer for protons (maintains intracellular pH level in the range of 6.8 - 7.0), heavy metals, and reactive oxygen species (ROS) [30, 31]. Preincubation of rat neurons with carnosine prevents ROS formation upon NMDA receptor

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Fig. (4). Dependency of ERK1/2 activation on the incubation time of rat cerebellar granule cells in the presence of 1 nM (A) or 1 μ M (B) ouabain. Kinase activation was evaluated by optical density of the bands related to the enzyme phospho-form, with reference to the value of the total pool of enzyme molecules and considering the value in intact cells as 100%. Other conditions as in Fig. (1).

activation, and at the same time a decrease in cell death is observed [32, 33].

At present, carnosine is being used in clinical trials as a complementary remedy in radiotherapy, in ischemic damage of the cerebrum, and in treating Parkinson's disease [34-36]. Despite the great number of works devoted to the studying of the protective action of carnosine, the precise mechanisms of its action are still unknown. In this work we studied carnosine's effect on ERK1/2 activation under oxidative stress conditions.

We exposed neurons to the actions of the oxidative stress inducers NMDA and HC. In both cases, incubation with these compounds resulted in ROS accumulation and ERK1/2 kinase activation. As ERK1/2 kinase activity is an essential factor in adaptive cell response, its regulation by NMDA receptors and Na/K-ATPase is very interesting. This regulation has been found to perform with the participation of intracellular free radicals; thus, we evaluated carnosine's effect on this process.

Fig. (5) shows that carnosine prevents ERK1/2 activation induced by the glutamate receptor agonists HC and NMDA. In previous work, we showed that under similar conditions carnosine prevents ROS formation induced in neurons by NMDA or HC [34]. This fact emphasizes the key role of ROS in ERK1/2 activation and the importance of carnosine as an intracellular modulator of the activity of this enzyme.

6. INTERACTION OF SIGNALING PATHWAYS OF **ACTIVATION IN CEREBRAL NEURONS**

An increase in the level of free radicals in neurons is characteristic of such neurodegenerative diseases as Alzheimer's disease, Parkinson's disease, stroke, etc. [37]. As a rule, these diseases are also accompanied by hyperhomocysteinemia, which may act as an inducer of recurring strokes or as a factor aggravating the neurodegenerative process. Simultaneously, increased activation of ERK1/2 is observed in such diseases [2, 3]. Progressive oxidative stress has a de structive effect on cell structures, often resulting in cell



Fig. (5). Effect of carnosine (5 mM) on phospho-ERK1/2 level under activation of NMDA receptor with 500 µM HC or 500 µM NMDA. ERK1/2 activation was evaluated by flow cytometry using monoclonal FITC-labeled antibodies against the phospho- form of the enzyme. FITC fluorescence value obtained for control cells was considered as 100%.

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death. A high level of HC has a toxic effect on both the nervous and immune systems of the body *via* NMDA receptors of the appropriate cells [15]. The precise mechanisms of this action, however, have still not been studied.

As mentioned above, evidence of the controversial role of ERK1/2 in the cell's life has now been obtained. Triggering of one or another signalling cascade can depend on both the nature of the inducer and the duration of signal. For example, the EGF effect on murine pheochromocytoma PC12 cells results in proliferation, and the NGF effect results in differentiation. The action of both factors is mediated by MEK/ERK system. At the same time, it has been shown that the signal from EGF is short-term, lasting about 1 min, whereas the signal from NGF is more permanent, lasting 1 hour ERK1/2 is usually transiently phosphorylated in response to the actions of neurotrophic factors or neurotransmitters (e.g. glutamate). Such ERK1/2 activation may be important in the processes of long-term potentiation and neuron cell survival. At the same time, some data exist that the "pathologic" long-lasting activation of ERK1/2 (in contrast to the normal short-term activation) has a proapoptotic effect [38, 39].

Different profiles of ERK1/2 activation can be observed under the actions of various ligands on NMDA receptors. In cultured murine embryonic cerebral cortex cells, HC is able to induce a long-lasting activation of ERK1/2 (with a duration of more than 1 hour), while the action of glutamate is characterized by a short-term ERK1/2 activation [40]. Such differences in the activation profile of this key protein kinase can be important for providing different cell responses to the external stimuli because different profiles of ERK1/2 activation have different effects on gene expression [41]. As shown in this work, the time profile of ERK1/2 activation under the action of NMDA was different from that observed under the action of HC (see Fig. 1), an observation that corresponds to the "pathologic" mode of its activation.

In the experiments with ouabain, we also observed two forms of ERK1/2 activation corresponding to the adaptive pathway (low concentration corresponding to inactivation of the ouabain-sensitive form of Na/K-ATPase) and the pathologic pathway being realized when both forms of the enzyme are inactivated by the cardiotonic steroid. In one of our previous works, different concentrations of ouabain were shown to have different effects on the level of apoptotic death of rat cerebellar granule cells [42]. Incubation of neuronal cells with 1 mM ouabain (completely inhibiting all isoforms of Na/K-ATPase) resulted in a twofold increase in cell death by apoptosis, whereas their incubation with 100 nM ouabain (affects ouabain-sensitive isoforms of the enzyme) decreased apoptotic cell death by half. Thus, it can be concluded that according to the profile of ERK1/2 activation, two different ways of cell signaling pathways resulted in two opposite results. In our experiments we used carnosine as an agent preventing both modes of ERK1/2 kinase activation. The importance of carnosine as a natural modulator of ERK1/2 kinase consists in the fact that it acts as a buffer for free radicals, decreasing excessive toxicity of pathological factors (excitotoxicity) but not preventing the ROS signaling function in neuronal cells [40]. Therefore, the data described above show that carnosine is involved not only in maintain-

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ing a stable low level of ROS in the cell, but also in the regulation of intracellular signal transmission in which ROS plays the role of the second messenger [22].

The results are summarized in the scheme (Fig. 6), which shows the main routes of interaction between membrane proteins (NMDA receptors and Na/K-ATPase) and the intracellular signaling pathways transmitting information to the neuronal genome.



Fig. (6). Scheme describing two modes of signal transduction from the outer neuronal membrane to the genetic apparatus. Activation of NMDA receptors (NMDARs) by NMDA or inhibition of Na/K-ATPase by low ouabain concentrations results in accumulation of Ca^{2+} and ROS inside the cells and in short-term activation of ERK1/2. Activation of NMDARs by HC or inhibition of Na/K-ATPase by high concentrations of ouabain induces a long-term activation of ERK1/2. The two types of ERK1/2 activation result in different gene expression and cellular outcomes. The calcium signal stimulating the ROS production and ERK1/2 activation is derived from NMDAR activation and/or from Na/K-ATPase are not defined.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest. 230 Current Aging Science, 2012, Vol. 5, No. 3

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PATIENT'S CONSENT

Declared none

REFERENCES

- Guyton KZ, Gorospe M, Kensler TW, Holbrook NJ. Mitogenactivated protein kinase (MAPK) activation by butylated hydroxytoluene hydroperoxide: implications for cellular survival and tumor promotion. Cancer Res 1996 1; 56(15): 3480-5.
- [2] Chu CT, Levinthal DJ, Kulich SM, Chalovich EM, DeFranco DB. Oxidative neuronal injury. The dark side of ERK1/2. Eur J Biochem 2004; 271(11): 2060-66.
- [3] Klafki HW, Lewczuk P, Kamrowski-Kruck H, et al. Measurement of ERK 1/2 in CSF from patients with neuropsychiatric disorders and evidence for the presence of the activated form. J Alzheimer's Dis 2009; 18(3): 613-22.
- [4] Zhu X, Lee HG, Raina AK, Perry G, Smith MA. The role of mitogen-activated protein kinase pathways in Alzheimer's disease. Neurosignals 2002; 11(5): 270-81.
- [5] Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. Biochim Biophys Acta 2010; 1802(4): 396-05.
- [6] Garthwaite J. NMDA receptors, neuronal development, and neurodegeneration. New York: Oxford University Press. 1994.
- [7] Alford S, Brodin L The role of NMDA receptors in synaptic integration and the organization of motor patterns. New York: Oxford University Press. 1994.
- [8] Morris RGM, Davis M. The role of NMDA receptors in learning and memory. 2nd ed. New York: Oxford University Press. 1994.
- [9] Bear MF, Malenka RC. Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 1994; 4(3): 389-99.
- [10] Fagni L, Bossu JL, Bockaert J. Inhibitory effects of dihydropyridines on macroscopic K currents and on the largeconductance Ca-activated K-channel in cultured cerebellar granule cells. Pflugers Arch 1994; 429(2): 176-82.
- [11] Gunasekar PG, Kanthasamy AG, Borowitz JL, Isom GE. NMDA receptor activation produces concurrent generation of nitric oxide and reactive oxygen species: implication for cell death. J Neurochem 1995; 65(5): 2016-21.
- [12] Urushitani M, Nakamizo T, Inoue R, Sawada H, Kihara T, Honda K, Akaike A, Shimohama S. N-methyl-D-aspartate receptormediated mitochondrial Ca overload in acute excitotoxic motor neuron death: a mechanism distinct from chronic neurotoxicity after Ca influx. J Neurosci Res 2001; 63(5): 377-87.
- [13] Hidalgo C, Núñez MT. Calcium, iron and neuronal function. IUBMB Life 2007 (4-5): 280-5.
- [14] Zigmond MJ. Triggering endogenous neuroprotective mechanisms in Parkinson's disease: studies with a cellular model. J Neural Transm Suppl. 2006; (70): 439-42.
- [15] Boldyrev AA. Molecular mechanisms of homocysteine toxicity. Biochemistry (Moscow) 2009; 74(6): 589-98.
- [16] Makhro V, Bulygina ER, Boldyrev AA. Effects of Homocysteine and Homocysteinic Acid on cerebellar granule cells. Neurochem J 2007; 1(2): 127-32.
- [17] Kim WK, Pae YS. Involvement of N-methyl-D-aspartate receptor and free radical in homocysteine-mediated toxicity on rat cerebellar granule cells in culture. Neurosci Lett 1996; 216(2): 117-20.
- [18] Boldyrev AA. Why Homocysteine Is a Risk Factor of Neurodegenerative Diseases. Neurochem J 2007; 1(1): 14-20.
- [19] Cantley LC. Structure and mechanism of the Na/K-ATPase. Curr Top Bioenerg 1981; 2: 201-37.
- [20] Monteith GR, Blaustein MP. Different effects of low and high dose cardiotonic steroids on cytosolic calcium in spontaneously active hippocampal neurons and incultured glia. Brain Res 1998; 795(1-2):325-40.

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- [21] Kometiani P, Li J, Gnudi L, Kahn BB, Askari A, Xie Z. Multiple signal transduction pathways link Na/K-ATPase to growth-related genes in cardiac myocytes. J Biol Chem 1998; 273: 15249-56.
- [22] Xie Z, Askari A. Na/K-ATPase as a signal transducer. Eur J Biochem 2002; 269: 2434-39.
- [23] Boldyrev AA, Bulygina ER, Carpenter D, Schoner W. Glutamate Receptors Communicate with Na/K-ATPase in Rat Cerebellum Granule Cells. J Molecular Neurosci 2003; 3(21): 213-22.
- [24] Karpova LV, Akkuratov EE, Bulygina ER, Boldyrev AA. Both Ouabain-Sensitive and Ouabain-Resistant Isoforms of Na/K-ATPase in Cerebellar Granule Cells Control MAP Kinase Activity. Membr Cell Biol 2008; 2(2): 156-60.
- [25] Bagrov AY, Shapiro JI, Fedorova OV. Endogenous cardiotonic steroids: physiology, pharmacology, and novel therapeutic targets. Pharmacol Rev 2009; 61(1): 9-38.
- [26] Streck EL, Matte C, Vieira PS, Rombaldi F, Wannmacher CM, Wajner M, Wyse AT. Reduction of Na/K-ATPase activity in hippocampus of rats subjected to chemically induced hyperhomocysteinemia. Neurochem Res 2002; 27(12): 1593-98.
- [27] Bersier MG, Pena C, de Lores Arnaiz RG. The expression of NMDA receptor subunits in cerebral cortex and hippocampus is differentially increased by administration of endobain E, a Na/K-ATPase inhibitor. Neurochem Res 2008; 33(1): 66-72.
- [28] Bulygina ER, Boldyrev AA. Demonstration of differences in the action of several metabotropic and ionotropic glutamate agonists on intracellular reactive oxygen species and the Na-pump. J Alzheimer's Disease 2004; 5: 6.
- [29] Bulygina ER, Lyapina LY, Boldyrev AA. Activation of glutamate receptors inhibits Na/K-ATPase of cerebellum granule cells. Biochem 2002; 67(9): 1001-5.
- [30] Boldyrev AA. Carnosine: biological role and prospects for use in medicine. Biochem 1992; 57(9): 1302-10.
- [31] Boldyrev AA, Severin SE. The histidine-containing dipeptides, carnosine and anserine: distribution, properties and biological significance. Adv Enzyme Regul 1990; 30: 175-94.
- [32] Abe R, Shimoseqawa T, Moriizumi S, et al. Lipopolysaccharide induces manganese superoxide dismutase in the rat pancreas: its role in caerulein pancreatitis. Biochem Biophys Res Commun 1995; 217(3): 1216-22.
- [33] Boldyrev AA, Johnson P, Wei Y, Tan Y, Carpenter DO. Carnosine and taurine protect rat cerebellar granular cells from free radical damage. Neurosci Lett 1999; 263(2-3):169-72.
- [34] Boldyrev AA, Stvolinsky SL, Fedorova TN, Suslina ZA. Carnosine as a natural antioxidant and geroprotector: from molecular mechanisms to clinical trials. Rejuv Res 2010; 13(2-3): 156-58.
- [35] Shen Y, Hu WW, Chen Z. Carnosine and diseases of central nervous system. Zhejiang Da Xue Xue Bao Yi Xue Ban 2007; 36(2): 199-203.
- [36] Perry G, Nunomura A, Hirai K, et al. Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases? Free Radic Biol Med 2002; 33(11): 1475-9.
- [37] Boldyrev A, Bulygina E, Makhro A. Glutamate receptors modulate oxidative stress in neuronal cells. Neurotox Res 2004; 6(7-8): 581-7.
- [38] Marshall CJ. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell 1995; 80(2): 179-85.
- [39] Poddar R, Paul S. Homocysteine-NMDA receptor-mediated activation of extracellular signal-regulated kinase leads to neuronal cell death. J Neurochem 2009; 110(3): 1095-06.
- [40] Segal RA, Greenberg ME. Intracellular signaling pathways activated by neurotrophic factors. Annu Rev Neurosci 1996; 19: 463-89.
- [41] Glotin AL, Calipel A, Brossas JY, Faussat AM, Treton J, Mascarelli F. Sustained versus transient ERK1/2 signaling underlies the anti- and proapoptotic effects of oxidative stress in human RPE cells. Invest Ophthalmol Vis Sci 2006; 47(10): 4614-23.
- [42] Karpova LV, Bulygina ER, Boldyrev AA. Different neuronal Na/K-ATPase isoforms are involved in diverse signaling pathways. Cell Biochem Funct 2010; 28(2): 135-41.