Published in the Slovak Republic
European Journal of Medicine
Has been issued since 2013.
E-ISSN: 2310-3434
2018, 6(2): 72-82

DOI: 10.13187/ejm.2018.2.72
www.ejournal5.com

Investigation of the Effect of Polyphenol Euphorbin on the Transport of L Glutamate and Calcium Channels to Synaptosomes of Rat Brain

N.N. Khoshimov [a, *], R.N. Rakhimov [a], G.B. Akhmedova [b], V.G. Azizov [c]

[a] The Institute of Bioorganic Chemistry named after A.S. Sadikov, Academy of Sciences of the Republic of Uzbekistan, Republic of Uzbekistan
[b] National University of Uzbekistan, Republic of Uzbekistan
[c] Namangan State University, Republic of Uzbekistan

Abstract

Background: The purpose of this study was to determine the effect of L glutamate and polyphenol euphorbin on the transport of NMDA-receptor mediators in rat's brain synaptosomes. This makes it possible to adjust the transport of antagonists and agonists NMDA-receptors brain synaptosomes in rats.

Methods: The study was carried out using the Weilers method. Synaptosomes were isolated from the brain of rats by a two-step centrifugation method. The entire isolation procedure was carried out at 4°C. To measure the amount of cytosolic Ca^{2+} synaptosomes were calculated by the Grinkevich equation.

Results: Increase in the concentration of $[Ca^{2+}]_{in}$ caused by L glutamate, primarily due to activation of membrane permeability, movement of Ca^{2+} into the cell and release of Ca^{2+} from intracellular stores. The two-phase L glutamate process of induced release of protons from synaptic vesicles of rat brain nerve terminals is correlated with a two-step increase in the concentration of calcium under the influence of L glutamate. Euphorbin competes with L glutamate for glutamate binding site of NMDA-receptors. L glutamate partially reduces the action of euphorbin, which may indicate that part of the external calcium comes under the influence of euphorbin also through the open L glutamate binding site and in place of calcium channels NMDA-receptors.

Conclusion: In these studies, it was found that euphorbin slightly increases the fluorescence and the level of $[Ca^{2+}]_{in}$, respectively, in the synaptic membranes compared with the control. The obtained results indicate a possible competition between euphorbin and L glutamate for the site of regulation of the opening of ion channels of NMDA-receptors. It was found that the effect of euphorbin responsible for the opening of calcium channels with other sites of NMDA-receptors against the background of magnesium ions, argiolobatin and nifedipine, a change in the level of $[Ca^{2+}]_{in}$ synaptosomes was not observed.

Keywords: NMDA-receptors, synaptosomes, L glutamate, Euphorbin.

1. Introduction

Calcium plays an important role in the process of releasing the neurotransmitter and performing the function of transferring excitation and inhibition of the brain nerves. All this is closely related to the movement of calcium ions in nerve cells (Hardingham et al., 2010).

* Corresponding author:
E-mail addresses: nozimka@inbox.ru (N. Khoshimov)

Calcium is a key signaling ion involved in many different intracellular and extracellular processes ranging from synaptic activity to cell-cell communication and adhesion. The exact definition at the molecular level of the versatility of this ion has made overwhelming progress in the past several years and has been extensively reviewed.

Calcium ions act as a universal intracellular messenger in the cells of all living organisms. In addition, calcium ions play a key role in the work of excitable cells. For example, in nerve cells, calcium ions play an important role in the secretion and transduction of the signal of neurotransmitters.

The concentration of intracellular Ca^{2+} in neurons is a homeostatic parameter and under physiological conditions the transmembrane calcium exchange is regulated by several mechanisms. On the one hand, Ca^{2+} concentration increases as a result of the discovery of ligand-controlled and potential-controlled calcium channels, and the release of Ca^{2+} bound by intracellular depots upon activation of IP3 or ryanodine receptors of the endoplasmic reticulum. On the other hand, the excess concentration of intracellular Ca^{2+} is counteracted by ATP-dependent mechanisms of Ca^{2+} "pumping" through the plasmolemma and sequestration in the endoplasmic reticulum, Ca^{2+}/Na^{+} transmembrane exchange and other buffer and/or Ca^{2+}-binding processes. Coordinated management of these mechanisms controls the level of $[Ca^{2+}]_{in}$, allowing it to fluctuate within certain limits and with a certain spatio-temporal pattern to provide a variety of Ca^{2+}-dependent processes of intracellular signal transduction.

The rise in intracellular calcium levels upon synaptic activity triggers the activation of several kinases critical for the induction and expression of LTP. These include the calcium/calmodulin-regulated protein kinases CaMKII and CaMKIV (Wayman et al., 2008), the cAMP-dependent protein kinase A (PKA) (Abel et al., 2008), PKC (Malinow et al., 1989; Saito et al., 2002) and MAPK/ERKs. A broad range of evidence from molecular, cellular, and transgenic animal studies established CaMKII as a key factor in LTP. Postsynaptic injection of CaMKII inhibitors or genetic deletion of a critical CaMKII subunit blocked the ability to generate LTP and impaired learning in mice (Malenka et al., 1989; Malinow et al., 1989; Silva et al., 1992).

Brain functions are manifested at specific synapses through release of neurotransmitters inducing a number of biochemical signaling events in postsynaptic neurons. One of the most prominent of these events is a rapid and transient rise in calcium levels. This local increase in calcium concentrations results in a number of short-term and long-term synapse-specific alterations. These include the insertion or removal of specific calcium channel subunits at or from the membrane and the post-translational modification or degradation of synaptic proteins (Catterall et al., 2008; Greer et al., 2008; Higley et al., 2008).

The violation of calcium homeostasis in nerve cells is accompanied by many brain diseases. For example, in cerebral ischemic strokes, an avalanche-like increase in the concentration of calcium in the cytoplasm of neurons plays a major role in the chain of pathological disorders that lead to cell death by apoptosis, which causes all processes occurring in ischemic brain tissue to be termed the “calcium hypothesis of ischemic cell death”.

Neurotransmitters are types of hormones in the brain that transmit information from one neuron to another. They are synthesized by amino acids. Neurotransmitters control the body's main functions: movement, emotional reactions, physical ability to feel pleasure and pain. The most famous neurotransmitters affecting the regulation of nerve receptors are L glutamate, serotonin, noradrenaline, dopamine, acetylcholine and GABA.

With calcium deficiency, the release of the neurotransmitter is blocked, the excitation and inhibition mechanisms are violated.

L glutamate in neurons can develop neurodegenerative processes associated with violation of Ca^{2+} regulation, which trigger intracellular signaling cascades leading to the death of neurons (Khodorov, 2004). It is known that the neurotoxicity of L glutamate is involved in the pathogenesis of such socially important neurological diseases as epilepsy, ischemic stroke, migraine, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease. In this regard, the study of the mechanisms of neurotoxic action of L glutamate and agonists of its receptors is one of the most topical directions in modern neuroscience.

Calcium homeostasis perturbations in neurodegenerative diseases. Perturbations in calcium homeostasis were observed in several neurodegenerative disorders including Alzheimer's disease (AD) (Mattson, 2004; Selkoe, 2001; Bezprozvanny et al., 2008; Green et al., 2008; Mattson, 2007),

Parkinson's disease (PD) (Thomas et al., 2007; Hallett et al., 2004; Surmeier,2007), Huntington's disease (HD) (Ramaswamy et al., 2007; Nakamura et al., 2007; Fan et all 2007; Bezprozvanny, 2007), and amyotrophic lateral sclerosis (ALS) (Rowland et al., 2001; Strong et al., 2005; Alexianu et al., 1994; von Lewinski et al., 2005). Calcium homeostasis disruption implicates several mechanisms, such as alterations of calcium buffering capacities, deregulation of calcium channel activities, or excitotoxicity. Rare examples support a direct causative role of calcium homeostasis deregulation in neurodegeneration. However, compelling evidence supported by an increasing number of publications on this topic, highlights the importance of calcium deregulation in the neurodegenerative process (Bezprozvanny, 2008; Wojda et al., 2008). We will focus in this section on how calcium homeostasis is affected in neurodegenerative disorders by taking non exhaustive examples in AD, PD, HD, and ALS.

In the brain, calcium is fundamental in the control of synaptic activity and memory formation, a process that leads to the activation of specific calcium-dependent signal transduction pathways and implicates key protein effectors, such as CaMKs, MAPK/ERKs, and CREB. Properly controlled homeostasis of calcium signaling not only supports normal brain physiology but also maintains neuronal integrity and long-term cell survival. Emerging knowledge indicates that calcium homeostasis is not only critical for cell physiology and health, but also, when deregulated, can lead to neurodegeneration via complex and diverse mechanisms involved in selective neuronal impairments and death. The identification of several modulators of calcium homeostasis, such as presenilins and CALHM1, as potential factors involved in the pathogenesis of Alzheimer's disease, provides strong support for a role of calcium in neurodegeneration. These observations represent an important step towards understanding the molecular mechanisms of calcium signaling disturbances observed in different brain diseases such as Alzheimer's, Parkinson's, and Huntington's diseases.

It should be noted that the vast majority of data on the effect of L glutamate on neurotransmission processes were obtained in electrophysiological experiments in which the main criterion for evaluating the effect of activation of presynaptic L glutamate receptors was the change in the frequency and amplitude of the registered synaptic currents in postsynaptic structures. The extremely small geometric dimensions of most nerve terminals are a serious obstacle to the successful conduct of direct measurements of the corresponding phenomena in presynaptic formations. In this regard, information on those intracellular processes developing in the presynaptic nerve structures was carried out using fluorescent probes.

The study of the mechanisms of calcium homeostasis regulation in excitable cells, the search for biologically active substances and physical factors that affect this homeostasis is one of the most urgent tasks of modern science.

Purpose: The effects of L glutamate and euphorbin polyphenol on the transport of NMDA-receptor mediators in synaptosomes rats brain.

2. Material and methods

Experiments were conducted on 20 outbred male albino rats weighing (200-250 g) contained in a standard vivarium ration. All experiments were performed in accordance with the requirements of "the World Society for the Protection of Animals" and "European Convention for the protection of experimental animals" (European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. 1986). Synaptosomes isolated from rat brain by a two-step centrifugation (Weiler et al., 1981). The whole procedure of selection was carried out at 4°C. To measure the amount of cytosolic Ca^{2+} was calculated from the equation of Grinkevich (Grynkiewicz et al., 1985) in synaptosomes isolated from brain of rats placed in an environment similar to, the one that was used to isolate cells were added 20 μM of chlortetracycline (CTC). Incubated for 60 min to achieve maximal interaction with the membrane - CTC Ca^{2+} as in plasma, and intracellular membranes. CTC excitation wavelength – 405 nm, recording – 530 nm. Results are expressed as a percentage, taking 100 % of the difference between the maximum value of fluorescence intensity (fluorescence dye, a saturated Ca^{2+}) and its minimum value (in the absence of fluorescence of the indicator of Ca^{2+}) obtained after adding ethylene-glycol-bis-amino-ethyl-tetra-acetate EGTA.

Statistical analysis

The measurements were made using a universal spectrometer (USB-2000). Statistical significance of differences between control and experimental values determined for a number of data using a paired t-test, where the control and the experimental values are taken together, and unpaired t-test, if they are taken separately. The value of P <0.05 indicated a statistically significant differences.

The results obtained are statistically processed to Origin 7,5 (Origin Lab Corporation, USA).

3. Results and discussion

Investigation of the effect of L glutamate on the level of cytoplasmic calcium in brain synaptosomes of rats.

Synaptosomes obtained from rat brain were used in the work, which is an adequate and convenient model for studying presynaptic processes. The activity of L glutamate was judged by the change in the intensity of the fluorescent signal, by the change in the cytoplasmic levels of free calcium $[Ca^{2+}]_{in}$.

A fluorescence ratio excited by light at 340 and 380 nm (F_{340}/F_{380}) in synaptosomes was established with the help of the Ca^{2+} -sensory chlortetracycline probe (CTC). When Ca^{2+} was removed from the extracellular medium, preincubation of EGTA resulted in a 10% decrease in fluorescence. In the presence of EGTA in the incubation medium (Figure 1), L glutamate in concentrations of (10-100 μM) dose-dependently increases the level of fluorescence by 15-25 %, which indicates an increase in $[Ca^{2+}]_{in}$ concentration caused by L glutamate, primarily due to activation of membrane permeability, displacement of Ca^{2+} into the cell and release of Ca^{2+} from intracellular depots (Figure 2).

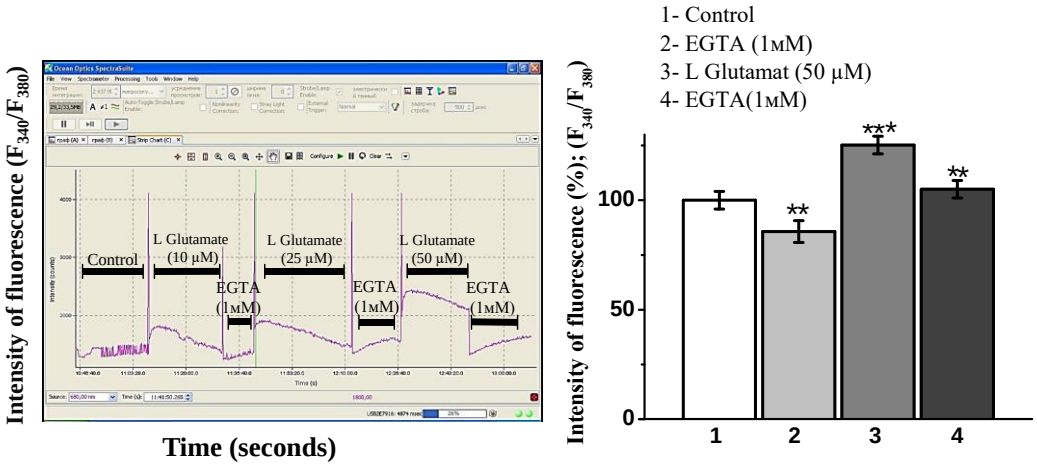

Fig. 1. Fluorescence intensity change with L glutamat (10-50 μM) when incubated with rats of brain tumor synaptosomes EGTA (1 mM). Increased fluorescence intensity induced by L glutamat (50 μM). Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

In the following experiments, it was shown that when 100 μM L glutamate was added to the synaptic suspension, a change in the intensity of the fluorescent signal was clearly indicated, which clearly indicated the two-phase nature of the process. With the addition of 100 mM L glutamate, the first ("fast") phase was a sharp increase in fluorescence intensity of the CTC (within 5-10 s), followed by its attenuation down to the initial level. The first phase of the response to the action of L glutamate was similar to that observed when 30 mM KCl was added. This effect, which leads to the depolarization of the plasma membrane, stimulates the process of exocytosis in the calcium containing medium. Depolarization of nerve endings in the presence of Ca^{2+} caused a rapid increase in $[Ca^{2+}]_{in}$, which occurred in two stages. The nature of the initial phase of the response to the action of L glutamate suggests that the primary response to the activation of L glutamate presynaptic receptors is the stimulation of the process of exocytosis.

After the completion of the first, a second, more "slow" phase began to develop, which was characterized by a gradual increase in the intensity of the fluorescent signal (Figure 3).

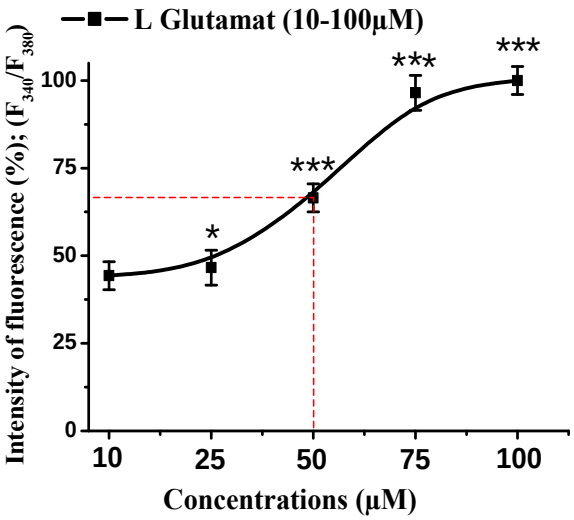

Fig. 2. The dose-dependent effect of L glutamate on the level of intracellular calcium in the brain synaptosomes of rats. Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

Analysis of the dose response of the effect of L glutamate revealed that the magnitude of only the first ("fast") phase of the response is directly proportional to the concentration of the agonist (Figure 3). With an increase in the L glutamate concentration to 100 μM, the amplitude of the "burst" of the fluorescent signal increased, which may be due to the involvement of more synaptic vesicles in the exocytosis process. An increase in the concentration of L glutamate in this case led to a decrease in the period lag between the two phases.

As a result, at an agonist concentration of 100 μM after a primary increase in fluorescence intensity, an inverse change in the fluorescent signal was not observed at all.

Since the nature of the development of the first phase of the response to the action of L glutamate suggested that this phase reflects the process of exocytosis, it was logically justified to try to clarify the role of calcium in the development of this process.

Thus, the two-phase process of L glutamate observed by us, induced release of protons from synaptic vesicles of rat brain nerve terminals, correlates with a two-step increase in the concentration of calcium under the influence of L glutamate.

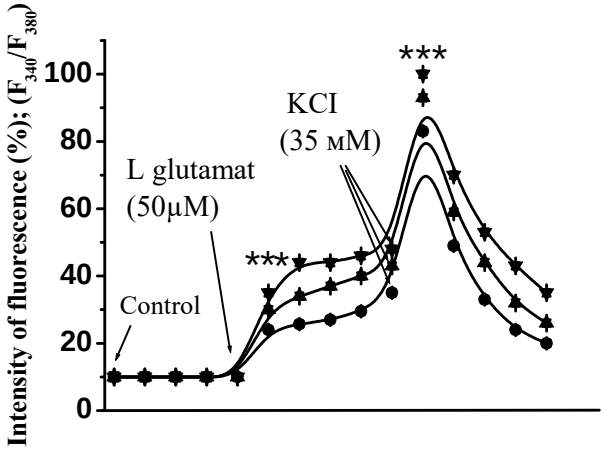

Fig. 3. Comparative effect of rats of the L-glutamate acid (50 μM) and KCl (35mM) on the fluorescence intensity of the rats synaptosomes suspension. Effect of L Glutamate (50 μM) and KCl (35 μM) solution on time-dependent fluorescence intensity. Ordinate axis - the intensity of fluorescence expressed in percent (%), the abstractions on the axis - time (min). Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 4).

A significant contribution to the maintenance of an elevated Ca^{2+} level in the cytosol can be caused by the activation of potential-dependent Ca^{2+} channels and the inversion of Na^{+}/Ca^{2+}-transmembrane exchange (Siesjo et al., 1989).

In addition to increasing the level of intracellular free Ca^{2+} due to entry from outside the cell, the processes of maintaining its high concentration in the cytosol due to the release of calcium from the membranes of the endoplasmic reticulum and mitochondria, as well as the disturbance of the processes of its sequestration, are of great importance.

It is known that the change in calcium transport by presynaptic membranes is accompanied by an increase in glutamatergic transmission, which is due to an increase in the release of L glutamate. Excitatory neurotransmitter L glutamate can cause damage and death of DA neurons, and therefore the damaging effect of glutamate on neurons is indicated by the term "toxicity of excitatory amino acids", or “excitotoxicity”.

The L glutamate excitotoxicity is mediated by NMDA-receptors, named for a specific N-methyl-D-aspartate antagonist. When the L glutamate interacts with these receptors, the ion channels of the neuronal membrane open and the L glutamate enters the neuron. The extensive binding of L glutamate with NMDA-receptors leads to an increase in the current of Ca^{2+} to the neuron through NMDA-receptor channels. Due to the fact that Ca^{2+} current amplification is one of the leading mechanisms of neuron death, it can be assumed that the mechanism of excitotoxicity of L glutamate in Parkinson's disease (BP) is associated with a massive entrance of Ca^{2+} into DA-neurons of a black substance. The violation of glutamatergic transmission is now also considered as a leading factor in the pathogenesis of diseases such as epilepsy, Alzheimer's disease, etc. (Choi, 1995; Stout et al., 1998; Nicholls et al., 2000; Vergun et al., 2001).

The effect of polyphenol euphorbin (*1-O-galloyl-6-bisgalloyl-2,4-valoneoyl-β-D-glucose*) isolated from the plant (*EUPHORBIA HIMUFUSA*) on the glutamatergic neurotransmitter system in rat brain synaptosomes was studied.

Preincubation of Euphorbin (10-100 μM) with the complex of the CTC-synaptosomes increases the fluorescence and accordingly, the level of $[Ca^{2+}]_{in}$ difference from L glutamate (Figure 4).

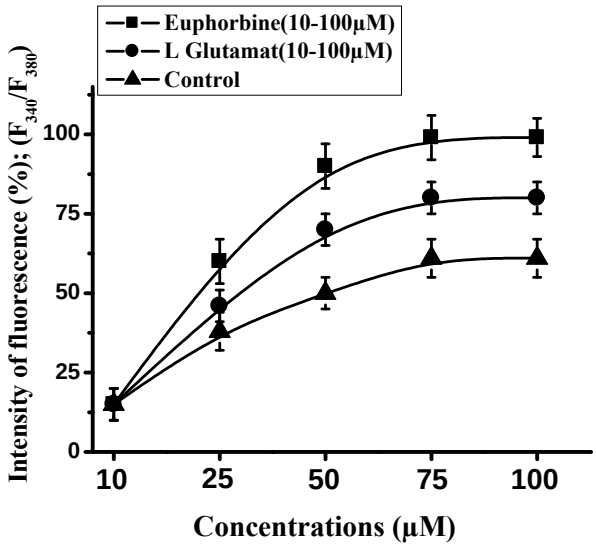

Fig. 4. Effects of Euphorbine and L glutamate in concentrations (10-100 μM) on the intensity of CTC fluorescence of brain synaptosomes suspension in rats. Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

Euphorbin (50 μM) reduced the fluorescence and accordingly the level of $[Ca^{2+}]_{in}$ against the background of L glutamate (50 μM) on the complex of CTC-synaptosomes (Figure 5).

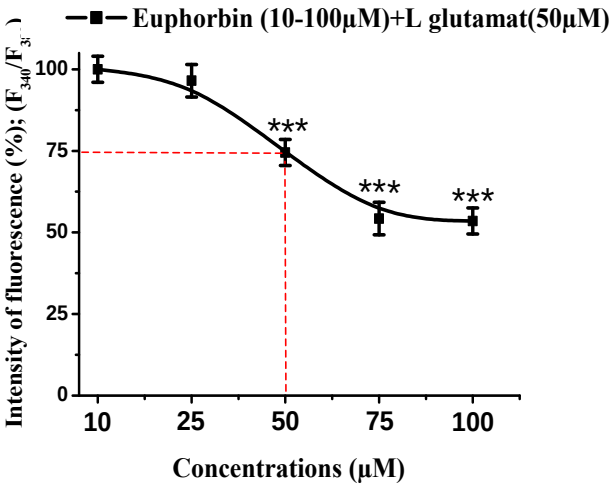

Fig. 5. Effect on fluorescence intensity in synaptosomes suspension in conditions of incubation with euphorbin (10-100 μM) L glutamate (50 μM). Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

The preliminary preincubation of euphorbin (10 μM) with synaptic membranes, then the addition of CTC- L glutamate resulted in a decrease in fluorescence and a level of $[Ca^{2+}]_{in}$, respectively. A dose-dependent increase in euphorbin concentration to (10-100 μM), respectively, resulted in a dose-dependent decrease in the effect of L glutamate (Figure 5).

The effect of L glutamate was observed depolarization of the synaptic membrane and an increase in intracellular calcium without an appreciable change in the concentration of internal sodium ions. Increase in synaptosomal calcium was inhibited by the addition of L glutamate. Activation of L glutamate receptors causes the opening of calcium channels ionotropic receptors, calcium influx into synaptosomes and depolarization of the synaptosomal plasma membrane, followed by the release of amino acid neurotransmitters.

L Glutamate partially reduces the action of euphorbin, which may indicate that part of the external calcium comes under the influence of euphorbine also through the open glutamine site and in place of calcium channels NMDA-receptors.

Even the preliminary addition of L glutamate does not completely abolish the action of euphorbin, which may indicate that euphorbin has several mechanisms of action on rat brain neurons, the result of which is an increase in $[Ca^{2+}]_{in}$.

From the literature data it is known that, Mg^{2+} ions selectively block the activity of NMDA-receptors. Glycine enhances NMDA-receptor responses by increasing the frequency of channel opening. In the complete absence of glycine, the receptor is not activated by L glutamate.

Indeed, the addition of glycine to the incubation medium (5 μM) enhanced the L glutamate-dependent increase in fluorescence by 15-22 %. At the same time, Mg^{2+} ions (50 μM) inhibited L glutamate-induced Ca^{2+} release from intracellular depots (Figure 6).

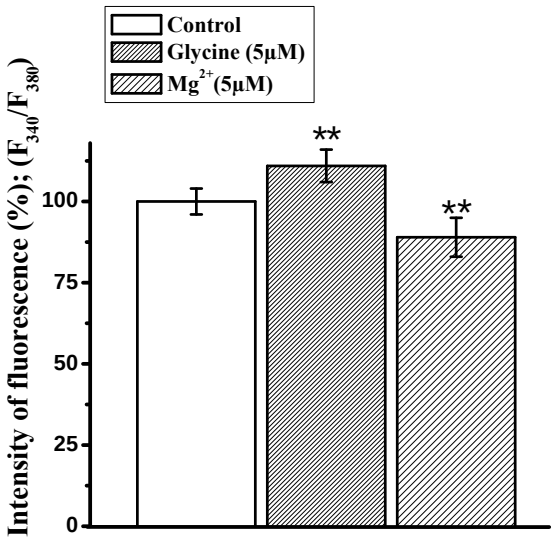

Fig. 6. Effect of glycine and Mg^{2+} ions on L glutamate-inducible Ca^{2+} intracellular depot. Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 5)

It is known that glycine stimulating effects of L glutamate and competitive receptor antagonists such as AP_5, AV-2-1 toxin can prevent activation of L glutamate. Other drugs and Mg^{2+} ions may block the open channel through the non-competitive antagonism. These medications include experimental neuroprotective drug MK-801 and argiolobatin (Martin et al., 1977).

In order to identify, possible interaction with polyphenol euphorbin areas over stimulation NMDA-receptor responsible for the opening of calcium channels, investigated its effect on the background of the non-competitive antagonists such as magnesium ions, argiolobatin and calcium channel blockers – nifedipine

It is shown that magnesium ions in millimolar concentrations significantly inhibit the fluorescence of the L glutamate-CTC-synaptosomes complex. The inhibitory effect of magnesium ions against the background of euphorbin (50 μM) of the fluorescence of the CTC-synaptosomes complex did not change.

In these studies, it was shown that in the presence of euphorbin, the inhibitory effect of magnesium ions (50 μM) was not observed. This is probably due to the fact that there is no competition between Mg^{2+} and euphorbin over sites that stimulate the opening of ion channels. It has also been shown that the action of argiolobatin (10 μM) on the calcium channels of the NMDA-receptor in the presence of euphorbin (50 μM) does not change (Figure 7).

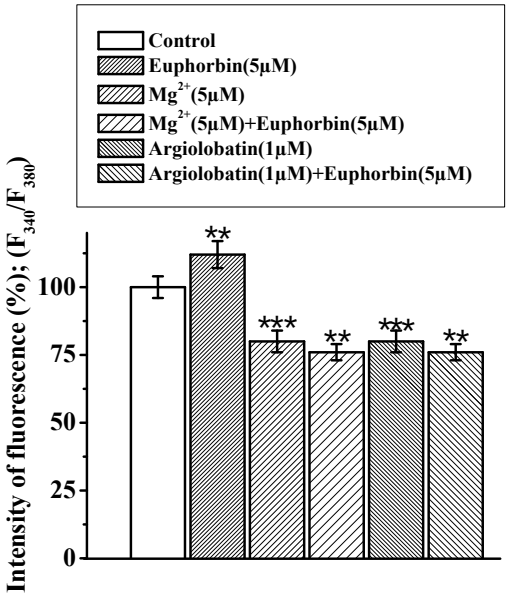

Fig. 7. Effect of non-competitive NMDA-receptor antagonists Mg^{2+} and argiolobatin on the background of euphorbin on fluorescence intensity and the level of $[Ca^{2+}]_{in}$ in the brain synaptosomes of rats. Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

When investigating the effect of euphorbin on calcium-dependent NMDA-receptor processes were studied against the background of the blocker of the L-type Ca^{2+} channels of nifedipine in the brain synaptosomes of rats.

Preincubation of nifedipine (0.01 μM) with the suspension complex of the CTC-synaptosomes resulted in a decrease in fluorescence. Preincubation of euphorbin (50 μM) with the suspension complex of the CTC-synaptosomes, no decrease in fluorescence. Preincubation of euphorbin (50 μM) against a background of nifedipine (0.01 μM) with a complex of CTC-synaptosomes did not result in a change in fluorescence (Figure 8), indicating that there is no competition between euphorbin and nifedipine for the site of regulation of dihydropyridine-sensitive calcium channels.

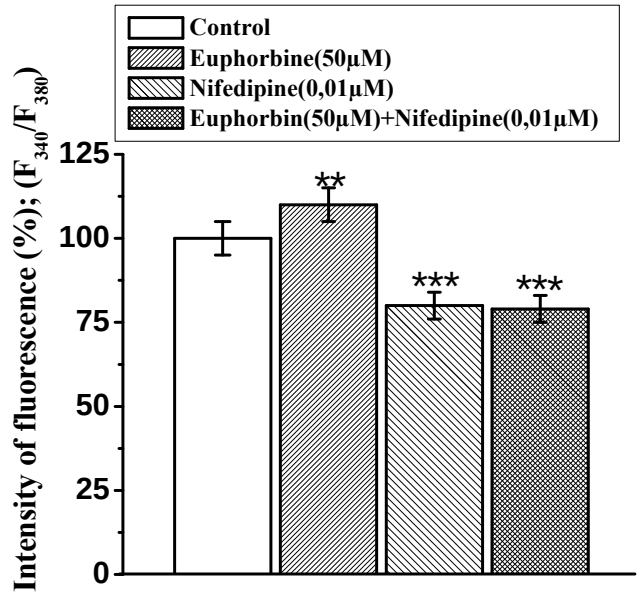

Fig. 8. Effect of euphorbin on calcium-dependent NMDA-receptor processes on the background of nifedipine. Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

This is explained by the fact that, euphorbin does not work for the site of regulation of the dihydropyridine-sensitive calcium channels of the rat brain synaptosomes membrane.

4. Conclusion

In these studies, it was found that euphorbin slightly increases the fluorescence and the level of $[Ca^{2+}]_{in}$, respectively, in the synaptic membranes compared with the control. The results obtained indicate a possible competition between euphorbin and L glutamate for the site of regulation of the opening of ion channels of NMDA-receptors.

It was found that the effect of euphorbin responsible for the opening of calcium channels with other sites of NMDA-receptors against the background of magnesium ions, argiolobatin and nifedipine, a change in the level of $[Ca^{2+}]_{in}$ synaptosomes was not observed.

The results indicate the possibility of using euphorbin, as an exciting neurotransmitter in neurodegenerative diseases.

References

Hardingham et al., 2010 – *Hardingham G.E., Bading H.* (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat Rev Neurosci.* V. 11(10). pp. 682–96.

Wayman et al., 2008 – *Wayman G.A, Lee Y.S, Tokumitsu H, Silva A, Soderling T.R.* (2008). Calmodulin-kinases: modulators of neuronal development and plasticity. *Neuron*, 59: 914–931.

Abel et al., 2008 – *Abel* T, *Nguyen P.V.* (2008). Regulation of hippocampus-dependent memory by cyclic AMP-dependent protein kinase. *Prog Brain Res.,* 169: 97–115.

Malinow et al., 1989 – *Malinow R., Schulman H., Tsien R.W.* (1989). Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science,* 245: 862–866.

Saito et al., 2002 – *Saito N, Shirai Y.* (2002). Protein kinase C gamma (PKC gamma): function of neuron specific isotype. *J Biochem.,* 132: 683–687.

Malenka et al., 1989 – *Malenka R.C., Kauer J.A., Perkel D.J., Mauk M.D., Kelly P.T., Nicoll R.A., Waxham M.N.* (1989). An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. *Nature.* 340: 554–557.

Silva et al., 1992 – *Silva A.J., Paylor R., Wehner J.M., Tonegawa S.* (1992). Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science,* 257: 206–211.

Catterall et al., 2008 – *Catterall W.A., Few A.P.* (2008). Calcium channel regulation and presynaptic plasticity. *Neuron*, 59: 882–901.

Greer et al., 2008 – *Greer P.L, Greenberg M.E.* (2008). From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. *Neuron,* 59: 846–860.

Higley et al., 2008 – *Higley M.J, Sabatini B.L.* (2008). Calcium signaling in dendrites and spines: practical and functional considerations. *Neuron,* 59: 902–913.

Khodorov, 2004 – *Khodorov B.* (2004). Glutamate-induced deregulation of calcium homeostasis and mitochondrial dysfunction in mammalian central neurons. *Progr. Biophys. Molec. Biol.,* V. 86. №. 2. pp. 279–351.

Mattson, 2004 – *Mattson M.P.* (2004). Pathways towards and away from Alzheimer's disease. *Nature*, 430: 631–639.

Selkoe, 2001 – *Selkoe D.J.* (2001). Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev.,* 2001, 81: 741–766.

Bezprozvanny et al., 2008 – *Bezprozvanny I., Mattson M.P.* (2008). Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci.,* 31: 454–463.

Green et al., 2008 – *Green K.N., LaFerla F.M.* (2008). Linking calcium to Abeta and Alzheimer's disease. *Neuron,* 59: 190–194.

Mattson, 2007 – *Mattson M. P.* (2007). Calcium and neurodegeneration. *Aging Cell,* 6: 337-350.

Thomas et al., 2007 – *Thomas B., Beal M.F.* (2007). Parkinson's disease. *Hum Mol Genet,* 16 (Spec №2): R183–194.

Hallett et al., 2004 – *Hallett P.J., Standaert D.G.* (2004). Rationale for and use of NMDA receptor antagonists in Parkinson's disease. *Pharmacol Ther,* 102: 155–174.

Surmeier, 2007 – *Surmeier D.J.* (2007). Calcium, ageing, and neuronal vulnerability in Parkinson's disease. *Lancet Neurol,* 6: 933–938.

Ramaswamy et al., 2007 – *Ramaswamy S, Shannon K.M, Kordower J.H.*: Huntington's disease: pathological mechanisms and therapeutic strategies. *Cell Transplant,* 16: 301–312.

Nakamura et al., 2007 – *Nakamura K, Aminoff M.J.* (2007). Huntington's disease: clinical characteristics, pathogenesis and therapies. *Drugs Today (Barc)*, 43: 97-116.

Fan et al., 2007 – *Fan M.M, Raymond L.A.* (2007). N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Prog Neurobiol,* 81: 272-293.

Bezprozvanny, 2007 – *Bezprozvanny I.* (2007). Inositol 1,4,5-tripshosphate receptor, calcium signalling and Huntington's disease. *Subcell Biochem,* 45: 323–335.

Rowland et al., 2001 – *Rowland L.P, Shneider N.A.* (2001). Amyotrophic lateral sclerosis. *N Engl J Med*, 344: 1688–1700.

Strong et al., 2005 – *Strong M.J., Kesavapany S., Pant H.C.* (2005). The pathobiology of amyotrophic lateral sclerosis: a proteinopathy? *J Neuropathol Exp Neurol,* 64: 649–664.

Alexianu et al., 1994 – *Alexianu M.E., Ho B.K., Mohamed A.H., La Bella V., Smith R.G., Appel SH.* (1994). The role of calcium-binding proteins in selective motoneuron vulnerability in amyotrophic lateral sclerosis. *Ann Neurol,* 36: 846–858.

von Lewinski et al., 2005 – *von Lewinski F, Keller B.U.* (2005). Ca^{2+}, mitochondria and selective motoneuron vulnerability: implications for ALS. *Trends Neurosci*, 28: 494–500.

Wojda et al., 2008 – *Wojda U., Salinska E., Kuznicki J.* (2008). Calcium ions in neuronal degeneration. IUBMB Life, 60: 575-590.

European Convention..., 1986 – "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes." Strasbourg. 1986. Available from: http://conventions.coe.int

Weiler et al., 1981 – *Weiler, M.H., Gundersen C.B. and Jenden D.J.* (1981). Choline uptake and acetylcholine synthesis in synaptosomes: Investigations using two differently labelled variants of choline. *Neurochem*. 36. pp. 1802–1812.

Grynkiewicz et al., 1985 – *Grynkiewicz G., Poenie M., Tsien R.Y.* (1985). 'A new generation of Ca^{2+}, indicators with greatly improved fluorescence properties'. J. *Biol. Chem*. Vol. 260, pp. 3440–3450.

Siesjo et al., 1989 – *Siesjo B.K., Bengtsson F.* (1989). Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression: a unifying hypothesis. *J Cereb Blood Flow Metab*. V. 9 (2). pp. 127-140.

Choi, 1995 – *Choi D.W.* (1995). Calcium: still center-stage in hypoxic-ischemic neuronal death. *Trends Neurosci*. V. 18. № 2. pp. 58–60.

Stout et al., 1998 – *Stout A.K., Raphael H.M., Kanterewicz B.I., Klann E., Reynolds I.J.* (1998). Glutamate-induced neuron death requires mitochondrial calcium uptake. *Nat. Neurosci.* V. 1. №. 5. pp. 366–373.

Nicholls et al., 2000 – *Nicholls, D.G., Budd S.L.* (2000). Mitochondria and neuronal survival. *Physiol. Rev.*, V. 80. № 1. pp. 315–360.

Vergun et al., 2001 – *Vergun, O., Sobolevsky, A.I., Yelshansky, M.V., Keelan, J., Khodorov, B.I., Duchen, M.R.* (2001). Exploration of the role of reactive oxygen species in glutamate neurotoxicity in rat hippocampal neurones in culture. *J. Physiol.* V. 531. № Pt1. pp. 147–163.

Martin et al., 1977 – *Martin W.R., Sloan J.W.* (1977). Pharmacology and classification of LSD-like hallucinogens. In W.R. Martin (Ed.), Drug addiction II (P. 305-368). New York: Springer-Verlag.