The American Journal of Medical Sciences and Pharmaceutical Research
(ISSN – 2689-1026)
Published: May 12, 2021 | **Pages:** 1-8
Doi: https://doi.org/10.37547/TAJMSPR/Volume03Issue05-01

IMPACT FACTOR
2021: 5. 64
OCLC - 1121105510

Study Of The Neuroprotective Properties Of Biologically Active Compounds

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ABSTRACT

The works show that, using fluorescent probes, it was used to study the effect of PC-8 on changes in the dynamics of the intracellular Ca^{2+} content in synaptosomes of the rat brain, depending on the site of the binding of glutamate on calcium channels by a specific mediator with glutamate. To measure the amount of cytosolic Ca^{2+} synaptosomes, we calculated using the Grinkevich equation. It has been shown that polyphenol PC-8 binds to the glutamate-binding site of NMDA receptors, so that the conductance for Ca^{2+} ions is reduced through a channel blocking the effect of polyphenol PC-8 can be explained. due to its binding to the competing action of the site, the binding of glutamate to NMDA receptors.

KEYWORDS

NMDA-receptors, synaptosomes, intracellular Ca^{2+} , glutamate.

INTRODUCTION

According to the forecasts of the World Health Organization (Geneva, 2017), hundreds of millions of people around the world suffer from neurological disorders. More than 6 million people die each year from strokes; More than 50 million people suffer from

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epilepsy worldwide. There are an estimated 47.5 million people worldwide with dementia, with 7.7 million new cases each year - Alzheimer's is the most common cause of dementia. So, according to rough estimates, Alzheimer's disease and Parkinson's disease today in the world suffer, respectively, about 20 million. No less alarming are the incidence rates of stroke and chronic cerebrovascular diseases.

At present, there is a steady increase in the number of diseases of the nervous system all over the world, which, due to their prevalence and consequences, are classified as socially significant - vascular diseases of the brain, neurodegenerative diseases, epilepsy, multiple sclerosis, traumatic brain injury and disability due to neurological diseases. Biologically active compounds isolated from plants, such as polyphenols and alkaloids, are considered as promising sources for the creation of effective pharmacological drugs for the treatment of diseases of the nervous system.

At the same time, polyphenol derivatives attract the attention of researchers in the field of medicinal chemistry due to the wide spectrum of biological activity found in them, which allows them to be considered as promising compounds for the development of potential drugs, including for the treatment of socially significant diseases, such as neurodegenerative disorders leading to dementia.

The pathogenesis of many neurodegenerative diseases is based on an imbalance between the systems of inhibition and excitation.

It is known that neurodegeneration is induced by hyperactivation of various types of glutamate receptors, followed by calcium dysregulation, which triggers intracellular signaling cascades leading to apoptotic neuron death. Ionotropic calcium-permeable N-methyl-D-aspartate (NMDA) glutamate receptors are expressed by neurons in all parts of the mammalian CNS. Research in recent years has shown that polyphenols can interact with the glutamate or glycine binding site of the NMDA receptor [1,2,3,4,5,6].

For example, our earlier study showed that a comparative analysis of Ca^{2+} responses to rutan, NMDA, and glutamate showed that in neurons of the cortex and cerebellum of rats, rutan induced rapid Ca^{2+} oscillatory responses, while NMDA and glutamate caused a "classic" gradual increase in the intracellular concentration of Ca^{2+}. Such a variety of Ca^{2+}responses in cells can be mediated by a different composition of glutamate receptors on the plasma membrane of neurons [7,8,9].

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

Thus, from a brief review of the data, it can be seen that, on the basis of neurodegenerative diseases, significant changes occur in the activity and functional activity of various systems, processes occurring at the level of biological membranes, ion channels, receptors and membrane enzymes [10,11,12]. Therefore, it is these membrane formations that are currently considered the most potential

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targets for the search and development of new therapeutic approaches for the prevention and treatment of neurodegenerative changes and related diseases.

The promising nature of this approach is evidenced by the success in the treatment of neurodegenerative diseases, in the achievement of which drugs, whose action is based on modifying the functions of ion channels and receptors, played an important role [10]. Among them, calcium channel modifiers should be noted, which, by modifying the channel activity or membrane potential, control the excitability of nerve and muscle cells. A certain role was also played by modifiers and modulators of the function of choline, GABA, and glutamate NMDA receptors. However, despite these advances, drugs that do not fully meet the requirements for them and have various serious side effects continue to be used to treat neurodegeneration and related complications.

In this regard, the search for and characterization of new compounds capable of preventing and correcting dysfunctions of biological membranes, ion channels, and receptors arising in neurodegenerative diseases is an extremely urgent problem of modern pharmacology and medicine.

Biologically active compounds of natural origin which are characterized by a wide variety of chemical structures and a wide range of pharmacological effects can be of particular assistance.

The aim of the study effect (the polyphenolic compound is usually referred to as (PC-8), isolated from the plant Rhus typhina. The mechanism of action of PC-8 on calcium homeostasis has been studied in more detail against the background of glutamate

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" [13]. Synaptosomes isolated from rat brain by a two-step centrifugation [14]. The whole procedure of selection was carried out at 4°C. To measure the amount of membrane-bound Ca^{2+} to synaptosomes placed in a medium similar to that used for isolating the cells, but without apyrase and $MgCl_2$, 20 μM of chlortetracycline (CTC) was added. Incubated 60 min. To achieve maximum interaction of CT with membrane-bound Ca^{2+}, both on the plasma and intracellular membranes. The excitation wavelength of CTC is 405 nm; the registration is 530 nm. The results were expressed as a percentage, taking as 100% the difference between the maximum value of the fluorescence intensity (the fluorescence of the dye saturated with Ca^{2+}) and its minimum value (fluorescence of the indicator in the absence of Ca^{2+}) obtained after the addition of EGTA.

To measure the amount of cytosolic Ca^{2+} $[Ca^{2+}]_{in}$ was calculated by the Grinkevich equation [15] in synaptosomes isolated from the brain of rats. To measure the free cytosolic Ca^{2+} synaptosomes (1 × 108 cells / ml), 4 μM acetoxymethyl ester Fura-2AM was loaded for 40 min at 37 ° C. In this case, the ether group separates from the dye molecules penetrated

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into the cytoplasm by the action of intracellular esterases, resulting in the formed anion Fura-2, which binds Ca^{2+}. After completion of the loading, the dye remaining in the medium was removed by double washing and centrifugation in a standard medium. In the experiments, the concentration of cells in the cell was 5×10^{6} cells / ml. Fluorescence excitation was induced at 337 nm, and fluorescence detection at 496 nm. The fluorescence of the dye (F_{max}) saturated with Ca^{2+} was determined by adding 50 μM digitonin to Fura-2AM loaded cells. F_{min} was determined by measuring the fluorescence intensity in a non-calcium medium, $F_{min} = [(F_{max}-F_{af}) / 3] + F_{af}$, where F_{af} is the autofluorescence of cells determined by adding 0.1 mM $MnCl_2$ to a synaptic loaded with Fura-2AM and treated with digitonin. Registration of changes in the dynamics of calcium in cells used USB-2000 spectrometer (Ocean optics, USA_2010)

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).

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, L glutamate in concentrations of (10-100 μM) dose-dependently increases the level of fluorescence by 30-48%, 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. In addition to increasing the level of $[Ca^{2+}]_{in}$ 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

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"toxicity of excitatory amino acids", or "excitotoxicity".

After that, we conducted experiments effect of PC-8 isolated from the plant Rhus typhina on the changes $[Ca^{2+}]_{in}$ synaptosomes in rat's brain.

Preincubation of PC-8 (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.

PC-8 (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. The preliminary preincubation of PC-8 (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 PC-8 concentration to (10-100 µM), respectively, resulted in a dose-dependent decrease in the effect of L glutamate (Fig.1.). 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 PC-8, which may indicate that part of the external calcium comes under the influence of PC-8e 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 PC-8, which may indicate that PC-8 has several mechanisms of action on rat brain neurons, the result of which is an increase in $[Ca^{2+}]_{in}$. In order to identify, possible interaction with polyphenol PC-8 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 Zn^{2+}, magnesium ions and argiolobatin.

It is shown that Zn^{2+} and magnesium ions in millimolar concentrations significantly inhibit the fluorescence of the L glutamate-CTC-synaptosomes complex. The inhibitory effect of Zn^{2+} and magnesium ions against the background of PC-8 (50 µM) of the fluorescence of the CTC-synaptosomes complex did not change. In these studies, it was shown that in the presence of PC-8, the inhibitory effect of magnesium ions (50 µM) was not observed. This is probably due to the fact that there is no competition between Zn^{2+}, Mg^{2+} and PC-8 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 PC-8 (50 µM) does not change (Fig.1.).

The concentration of $[Ca^{2+}]_{in}$. in neurons - synaptosomes is a homeostatic parameter and, under physiological conditions, transmembrane calcium metabolism is regulated by several mechanisms. On the one hand, the concentration of Ca^{2+} increases as a result of the opening of ligand-gated and

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voltage-gated calcium channels, and the release of Ca^{2+} bound by intracellular stores, upon activation of IP3 or ryanodine receptors of the endoplasmic reticulum.

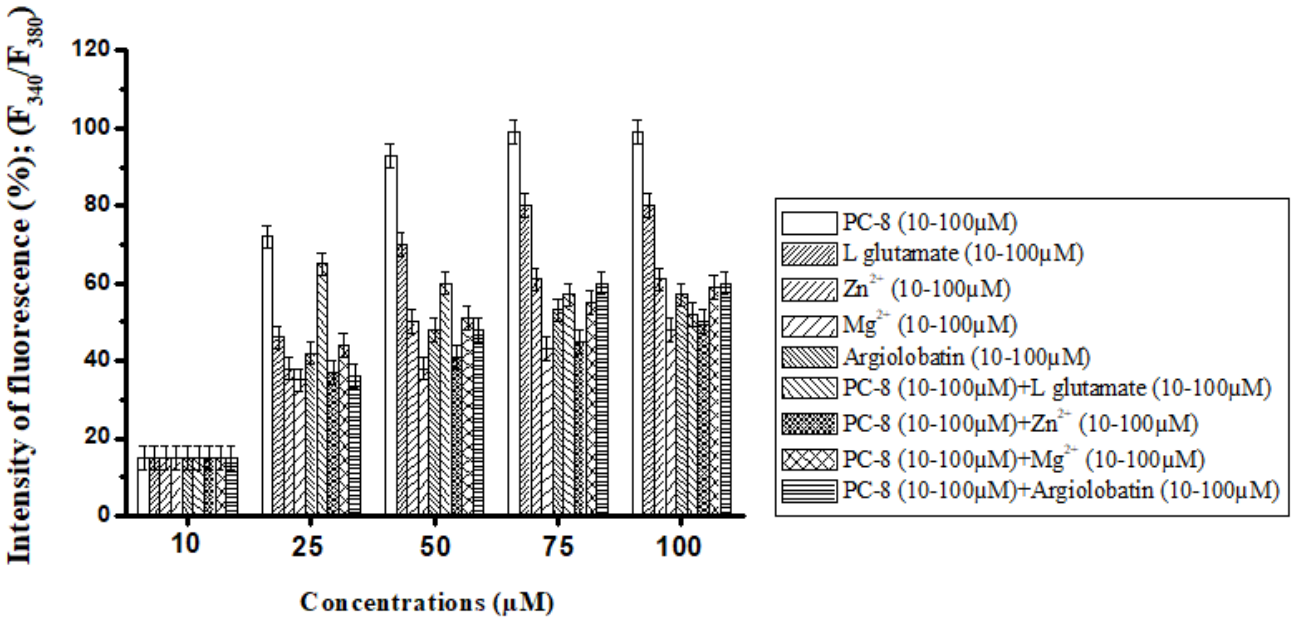

Figure. 1. The effect of PC-8 against the background of competitive and non-competitive antagonists such as glutamate, Zn^{2+}, Mg^{2+} and argiolobatin on the change in the concentration of $[Ca^{2+}]_{in}$ synaptosomes through the activation of NMDA-receptors.

On the other hand, an excessive concentration of intracellular Ca^{2+} is counteracted by ATP-dependent mechanisms of Ca^{2+} "pumping out" through the plasmolemma and sequestration in the endoplasmic reticulum, Ca2+/Na+ transmembrane metabolism and other buffer and / or Ca^{2+} binding processes. Coordinated control of these mechanisms controls the level of Ca^{2+} in the cytosol, allowing it to fluctuate within certain limits and with a certain spatio-temporal pattern to ensure a variety of Ca^{2+} dependent processes of intracellular signal transduction.

CONCLUSION

In these studies, it was found that PC-8 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 PC-8 and L glutamate for the site of regulation of the opening of ion channels of NMDA-receptors.

ACKNOWLEDGEMENT

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The work was supported by the Applied Research Program of the Ministry of Innovation of the Republic of Uzbekistan (project PZ-2017092060 - "Development of a heparin-like anticoagulant based on sulfated polysaccharides") No conflict of interest was declared.

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