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The Mechanism Of Action Of Polyphenol On Changes In The Dynamics Of Calcium In The Synaptosomes Of The Rat Brain Against The Background Of Glutamate

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ABSTRACT

The manuscript shows a short data used using fluorescent probes to study the effect of polyphenol PC-7 on changes in the dynamics of intracellular Ca^{2}+ content in rat brain synaptosomes, depending on the site of glutamate binding 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-7 binds to the β1-subunit of the voltage-gated calcium channel and allosterically changes its conformation so that the conductivity for Ca^{2}+ ions increases through the channel, the blocking effect of polyphenol PC-7 can be explained by its binding to voltage-gated calcium channels and activating them.

KEYWORDS

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

INTRODUCTION

Glutamate is the most common mediator in the brain and activates six different classes of receptors, three of which are ionotropic receptors [1]. Each class has its own pharmacological and functional features. There were no significant differences in the pharmacological properties of pre- and postsynaptic glutamate receptors. According to the names of agonists that cause specific physiological responses, N-Methyl-D-aspartate (NMDA) receptors, a-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid (AMPA) and

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kainate are isolated. All three classes of glutamate receptors are found in the central nervous system, and in many parts of the brain, the receptors coexist.

Two main types of glutamate receptors (ionotropic and metabotropic) include three functionally distinct receptor classes. Each of the receptors in the class consists of individual subunits encoded by different genes. Subunits of ionotropic glutamate receptors form ion channels that are permeable to cations. Metabotropic glutamate receptors are G-protein-linked receptors, the activation of which regulates the synthesis or degradation of intracellular secondary mediators [1,2,3,4,5,6,7,8].

Receptors differ in the composition of subunits and affinity for glutamate - for NMDA it is 1 μM. The functional significance of postsynaptic glutamate receptors is the transfer of excitation between neurons, whereas presynaptic receptors modulate the release of glutamate and other central nervous system mediators. The ligand-activated channel is a tetrameric complex; various combinations of subunits form functionally distinct receptors. The transmembrane topology of the glutamate receptor differs from that of the nicotinic receptor. The polypeptide contains three transmembrane domains M_1, M_2 and M_4 [2,9,10,11,12,13]. The NH_2-end is directed extracellularly; the COOH-end is intracellular. The M_2 domain forms a loop-pin, which forms the internal pore of the channel. The configuration is similar to the K-channels, which implies the common origin of the two classes of ion channels. Ionic channels of glutamate receptors are permeable to Na and Ca ions, but ionic selectivity depends on the subunit composition of the receptor [2,14,15,16]. Because of the specific nature of nerve cells, a fatal role in them is played by an increase in the concentration of Ca^{2+} in the presynaptic region, which leads to a massive release of various neurotransmitters, among which the exciting mediator glutamic acid has a special role. This is due to the fact that, first of all, the most synapses in the brain (about 40%) are glutamatergic and, secondly, the majority of postsynaptic glutamate receptors (NMDA- and AMPA-receptors) control the calcium channels and, accordingly, further increase the concentration Intracellular Ca^{2+}. In connection with this positive feedback, the concentration of cytoplasmic Ca^{2+} in glutamate-sensitive neurons increases dramatically. Therefore, up to the present time one more name of the processes occurring in the ischemic focus of the brain continues to exist: “exciting toxicity” or otherwise “glutamate excitotoxicity”.

The NMDA-receptor is one of the most finely regulated receptors: there are at least six binding sites for endogenous ligands that affect the probability of opening an ion channel. These include activating binding sites with two different agonists, glutamate and glycine, a polyamine binding site, and sites for binding to Mg, Zn, and H. The oxidative-reduction status of the receptor also affects conductivity. One of the three pairs of cysteine residues can be both restored (which increases the currents through the channel) and is oxidized with the formation of disulfide bridges (which reduces the currents).

The unique nature of the NMDA-receptor is that its activation requires simultaneous binding of two different agonists, so glutamate and glycine are called "coagents" of the NMDA-receptor. Low molecular weight glycine

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analogs, including serine and alanine, also act as agonists of the glycine site. In both cases, D-isomers are more effective than L-forms. So D-serine, formed by serine racemase, is a strong endogenous agonist of the glycine site. Extracellular Mg ions block the current through the NMDA-receptor channel. The magnesium block is removed when the membrane is depolarized. Protons reduce the frequency of opening the channel, and at pH ~ 6.0, the activation of the receptor is completely suppressed. Binding of polyamines - spermine or spermidine - removes the proton block and, thus, enhances the activation of the receptor. At high concentrations, polyamines, however, also cause a potential-dependent ion channel block [17,18,19,20].

In the central nervous system, Ca^{2+} is a ubiquitous cell messenger that participates in the regulation of neurotransmitter release and nerve excitability, and is closely associated with cellular differentiation and migration, synaptic plasticity, neurite growth, and neuronal apoptosis [21,22,23].

Previous studies have shown that in the experiments carried out, the effect of various polyphenols on the change in the dynamics of calcium in the synaptosomes of the rat brain, polyphenols act as antioxidant and neuroprotective properties [24,25,26,27,28,29,30].

Based on this research, in our next scientific experiments, in which synaptosomes of the rat brain were used, loaded with a probe on the cytosolic Ca^{2+} -Fura-2-AM, it was shown that in physiologically significant concentrations of 10^{-7}-10^{-5}M on the concentration of cytosolic calcium affects (the polyphenolic compound is usually referred to as (PC-7), isolated from plants Rhus typhina). The mechanism of action of PC-7 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" [31]. Synaptosomes isolated from rat brain by a two-step centrifugation [32]. 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 [33] 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

In our experiments with synaptosomes loaded with a fluorescent probe on Ca^{2+}, PC-7 increases the concentration of intracellular Ca^{2+} in a dose-dependent manner, both in the calcium-free and calcium-containing media. The action of PC-7 in a noncalcium medium indicates that it stimulates the release of calcium from intracellular compartments. Glutamate reduces the effect of PC-7, which may indicate that part of the external calcium comes under the influence of PC-7 through potential-sensitive calcium channels (Fig.1.A, B).

The effects of glutamate have been studied in rat brain synaptosomes. The effect of glutamate was observed depolarization of the synaptic membrane and an increase in intracellular calcium without a noticeable change in the concentration of internal sodium ions. An increase in the synaptosomal calcium and a decrease in the electrical potential of transmembrane cells was prevented by the addition of glutamate. Activation of the glutamate receptor causes the opening of calcium channels, the influx of calcium into the synaptosomes, and the depolarization of the synaptosomal plasma membrane, followed by the release of amino acid neurotransmitters (Fig.1.C).

Glutamate partially reduces the effect of PC-7, which may indicate that part of the external calcium comes under the influence of PC-7 also through the open glutamine site and in place of the calcium channels of the NMDA-receptors.

Even the preliminary addition of glutamate does not completely eliminate the effects of PC-7, which may indicate that PC-7 has several mechanisms of action on rat brain neurons, the result of which is an increase in intracellular calcium (Fig.1.D).

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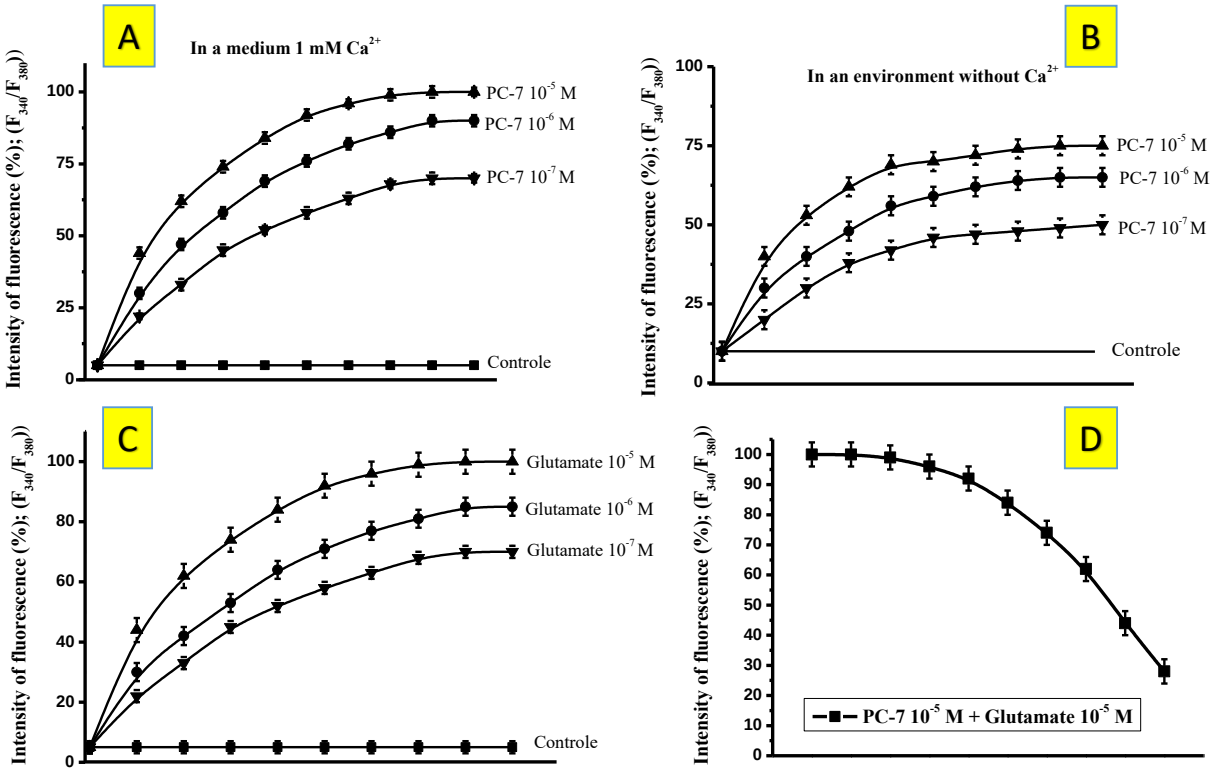

Figure. 1. The effect of PC-7 and glutamate on the fluorescence of synaptic membranes from the brain of rats pre-loaded with Fura-2. In a calcium-free and calcium-containing medium.

The ordinate shows fluorescence in percent, taking as 100% the difference between the maximum fluorescence intensity value in the presence of 1 mM Ca^{2+}, 25 μM digitonin and 1 mM rotenone and its minimum value when 1 mM EGTA is added to this medium.

Thus, our experiments in conjunction with the data indicate that PC-7 binds to the α_1- subunit of the potential-controlled calcium channel and allosteric changes its conformation so that the conductivity for Ca^{2+} ions increases through the channel.

In the medium with calcium, its effect is somewhat higher, but this excess is eliminated in the presence of glutamate. The effective concentrations of PC-7 are in the range 10^{-6}-10^{-5}M. Thus, the blocking effect of PC-7 can be explained by its binding to potential-controlled calcium channels and their activation.

CONCLUSIONS

- In this study, calcium chloride changes in synaptosomes were used to study, by means of fluorescent calcium probes, depending on the site of glutamate binding on calcium channels by specific mediators with glutamate. The results indicate a PC-7 binds to the α_1- subunit of a potential-controlled calcium channel and allosteric changes its conformation so that through the channel the conductivity for Ca^{2+} ions increases, blocking effect of PC-7 can be explained by its binding to potential-

controlled calcium channels and their activation

- As in any field of fundamental science, the completion of one stage does not solve all problems. In what areas can we continue to develop the results and conclusions of this paper. First, it is possible to continue screening among BASs of new substances acting on potential-controlled calcium channels. Secondly, it is necessary to continue the study of substances for which effects on these channels are found: polyphenols and alkaloids.

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