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ACTION OF CP-8 ON CA2+-CHANNELS OF RAT BRAIN SYNAPTOSOMES

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ABSTRACT: In the study, rat brain synaptosomes were used, which are an adequate and convenient model for studying presynaptic processes. L Glutamate activity was judged by the change in the intensity of the fluorescent signal, by the change in the cytoplasmic levels of free calcium [Ca2+]in. The study was conducted according to the Weiler method. Synaptosomes were isolated from rat brain using two-stage centrifugation. The entire isolation procedure was carried out at 4°C. To measure the amount of cytosolic Ca2+ synaptosomes were calculated according to the Grinkevich equation. An increase in the concentration of [Ca2+]in caused by L glutamate, primarily due to the activation of membrane permeability, the movement of Ca2+ into the cell,

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and the release of Ca2+ from intracellular depots. PC-8 competes with L glutamate for the glutamate binding site of NMDA-receptors.

KEYWORDS: NMDA-receptors, synaptosomes, L glutamate, PC-8.

INTRODUCTION

Glutamate is the most common mediator in the brain and activates six different classes of receptors, three of which are ionotropic receptors. 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 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].

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 [2].

The concentration of intracellular Ca2+ in neurons is a homeostatic parameter and under physiological conditions the transmembrane calcium exchange is regulated by several mechanisms. On the one hand, Ca2+ concentration increases as a result of the discovery of ligand-controlled and potential-controlled calcium channels, and the release of Ca2+ bound by intracellular depots upon activation of IP3 or ryanodine receptors of the endoplasmic reticulum. On the other hand, the excess concentration of intracellular Ca2+ is counteracted by ATP-dependent mechanisms of Ca2+ "pumping" through the plasmolemma and sequestration in the endoplasmic reticulum, Ca2+/Na+ transmembrane exchange and other buffer and / or Ca2+-binding processes. Coordinated management of these mechanisms controls the level of [Ca2+]in, allowing it to fluctuate within certain limits and with a certain spatio-temporal pattern to provide a variety of Ca2+-dependent processes of intracellular signal transduction.

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.

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Ca2+is a ubiquitous intracellular messenger that controls diverse cellular functions but can become toxic and cause cell death. Selective control of specific targets depends on spatio-temporal patterning of the calcium signal and decoding it by multiple, tunable and often strategically positioned Ca2+sensing elements. Ca2+ is detected by specialized motifs on proteins, which have been biochemically characterized decades ago. However, the field of Ca2+sensing has been reenergized by recent progress in fluorescent technology. These approaches exposed local Ca2+ sensing mechanisms inside organelles and at the organellar interfaces, revealed how Ca2+ binding might work to open some channels, and identified disorders linked to a variety of Ca2+ sensing proteins. We here, attempt to place these new developments in the context of intracellular calcium homeostasis and signaling [3].

The operation of Ca2+-signalling systems is constantly under review by an internal quality-assessment mechanism that can respond to changes in the properties of its output signal. We propose the hypothesis that Ca2+ itself has an important function in this internal assessment mechanism by remodelling its own signalling pathway. Several important disease states (hypertension, heart disease, diabetes, manic depression, Alzheimer's disease) might result from abnormal remodelling of Ca2+ signalling.

Based on this, our goal is to study the effect of PC-8 on calcium transport to synaptosomes in the rat brain.

Material and methods: Experiments were conducted on 20 outbred male albino rats weighing (250-300 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" [4]. Synaptosomes isolated from rat brain by a two-step centrifugation. The whole procedure of selection was carried out at 4°C [5]. To measure the amount of cytosolic Ca2+ was calculated from the equation of Grinkevich [6] 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 Ca2+ 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 Ca2+) and its minimum value (in the absence of fluorescence of the indicator of Ca2+) obtained after adding ethylene-glycol-bis-amino-ethyl-tetra-acetate EGTA.

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 [Ca2 +]in.

A fluorescence ratio excited by light at 340 and 380 nm (F340/F380) in synaptosomes was established with the help of the Ca2+ -sensory chlortetracycline probe (CTC). When Ca2+ 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

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an increase in [Ca2+]in concentration caused by L glutamate, primarily due to activation of membrane permeability, displacement of Ca2+ into the cell and release of Ca2+ from intracellular depots. In addition to increasing the level of [Ca2 +]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 "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 [Ca2+]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 [Ca2+]in difference from L glutamate.

PC-8 (50 µM) reduced the fluorescence and accordingly the level of [Ca2+]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 [Ca2+]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. 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 [7]. 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 [Ca2+]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 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 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 Mg2+ and PC-8 over sites that stimulate the opening of ion channels [8]. It

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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.

In these studies, it was found that PC-8 increases the fluorescence and the level of [Ca2+]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.

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