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Study of The Effect of Polyphenol Plantagin On Calcium Transport in The Presence of NMDA Receptor Agonists and Antagonists

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Abstract: This study investigated the effect of Plantagin polyphenol on NMDA receptor binding sites using glycine, Mg2+ and Zn2+ ions. A suspension of rat brain synaptosomes was isolated by differential centrifugation. Changes [Ca2+]in concentration in the suspension medium of rat brain synaptosomes were measured using the fluorescent probe Fluo-4 AM. It was noted that synaptosomal Ca2+ transport remained virtually unchanged under the influence of plantago polyphenols in Zn2+ and Mg2+ in rat brain synaptosomal suspensions incubated with L-glutamate-Fluo-4 AM. The polyphenol Plantagin competes with glycine for binding to the glycine site of NMDA receptors, reducing the likelihood of full receptor activation even in the presence of glutamate, which leads to a limitation of Ca^{2+} influx, and therefore plays an important role in preventing excitotoxicity.

Keywords: Plantagin polyphenol, NMDA receptor, Mg2+, Zn2+, synaptosome.

Introduction: NMDA receptors are present in all parts of the central nervous system, located mainly in the postsynaptic and partly in the presynaptic membrane [1]. NMDA receptors have also been found to function in astrocytes in the cerebral cortex [2].

The structure of the NMDA receptor consists of a complex of 4 separate basic subunits that form an ion channel in the central part that is permeable to Ca2+ ions. NMDA receptor subunits differ in their physiological and pharmacological properties, and seven subunits have been identified within the complex, including NR1, NR2A–D, NR3A, and NR3B. The NR1 subunit, which binds to glycine, has 8 isomeric forms in the NMDA receptor structure, and the NR2A–D subunit, which binds to 1 or 2 glutamic acid and provides the receptor with the function of an ion channel, are the functionally basic structures [3,4]. It is suggested that the NR3A and NR3B subunits expand the spectrum of functional properties of the NMDA receptor [5,6].

In clinical and experimental neurology, divalent cations such as Mg2+ and Zn2+ are widely used as NMDA receptor modulators [6,7].

Mg2+ ions, which are usually considered endogenous modulators, have been found to be more likely to bind to a specific site of the NMDA receptor at a highly negative membrane potential. Zn2+ ions bind to the GluN2A/GluN2B subunits [7].

It is known that magnesium ions play an important role in regulating the activity of NMDA receptors. They are potential-dependent receptor blockers, preventing excessive calcium influx and reducing excitotoxicity. Adequate amounts of Mg2+ are necessary for normal NMDA receptor function and synaptic plasticity. Zinc (Zn2+) ions can also modulate the activity of NMDA receptors. They act as positive allosteric modulators, enhancing the response of glutamate receptors. However, excessive levels of Zn2+ can lead to neurotoxicity and dysfunction of NMDA receptors.

NMDA receptors are heteromeric tetrameric proteins

composed of GluN1 and GluN2 subunits, which contain D-serine/glycine and glutamate binding sites, respectively.

In addition to D-serine/glycine and glutamate binding sites, they have several regulatory sites sensitive to polyamines, Zn2+, protons and glutathione [2,7,8,9].

It has been found that Mg2+ and Zn2+ ions have an inhibitory effect on NMDA receptors. These ions can block the NMDA receptor channel and reduce its activation by the neurotransmitter glutamate. Studies show that the inhibitory effects of Mg2+ and Zn2+ on NMDA receptors are not the same. It has been found that the blocking of the channel by Zn2+ is faster than by Mg2+.

In addition, the effect of Mg2+ and Zn2+ ions on NMDA receptors may depend on changes in pH. Therapeutic approaches identified through studies of the effects of Mg2+ and Zn2+ on NMDA receptors suggest that by regulating Mg2+ and Zn2+ levels, NMDA receptor activity can be modulated and neurological diseases associated with NMDA receptor dysfunction, such as Alzheimer's disease and epilepsy, can be prevented and treated.

The aim of the study was to determine the effect of the polyphenol Plantagin on NMDA receptor sites using glycine, Mg2+ and Zn2+ ions.

METHODS

Method for isolating rat brain synaptosome

In the studies, rat brain synaptosome suspensions were isolated by differential centrifugation using the method developed and modified by C.W. Cotman [10] [11].

The rat was sacrificed by dislocation, the cranial cavity was opened surgically, and the brain was removed. The brain preparation was homogenized in an incubation medium (pH=7.4) containing sucrose (0.32 M), Tris-HCl (0.01 M) and EDTA (0.5 mM) in a ratio of 1:10 under ice water conditions. In experiments, rat brain synaptosomes were isolated using 2/4-step centrifugation [12]. The first centrifugation was carried out at a speed of 4500 rpm and a duration of 10 minutes, and the obtained supernatant was centrifuged at a second stage at a speed of 14000 rpm and a duration of 20 minutes.

In the experiments, a KCl solution (35 mM) was used as a plasma membrane depolarizing agent. It is known that under incubation conditions with KCl (35 mM), membrane depolarization occurs and, in turn, activation of Ca2+ channels is observed [11]. In the synaptosome, the process of neurotransmitter secretion from the vesicle occurs due to an increase in the concentration of [Ca2+]in due to the entry or exit of Ca2+ ions from the SR through Ca2+ channels located in the presynaptic membrane [13].

Method for studying changes [Ca2+]in concentration in rat brain synaptosomes.

The change [Ca2+]in concentration in the suspension medium of rat brain synaptosomes was calculated using the method developed by Grynkiewicz et al. [14].

To determine the intracellular calcium concentration (1×108 cl/ml) in synaptosomes, the highly sensitive fluorescent probe Fluo-4 AM was used.

In our experiments, 1 mg of Fluo-4 AM powdered fluorescent probe was dissolved in 135 µL of DMSO to obtain 1 mM Fluo-4 AM reagent solution. Before the experiment, the Fluo-4 AM solution in DMSO was kept at room temperature [15] and 80 µl synaptosomes and 12 µl Fluo-4 AM were added to 2 ml Krebs-Ringer buffer and incubated for 30 min at 37°C. Fluo-4 AM is a fluorescent Ca2+ chelator with high affinity for calcium. Fluo-4 AM can specifically detect intracellular calcium ions with high sensitivity, low cytotoxicity and high content of acetyl methyl ester AM, which has good intracellular penetrating ability. After cleavage by intracellular esterase, it remains in the cell, binding to calcium ions and causing strong fluorescence.

In the experiments, the fluorescence intensity was recorded using a USB 2000 spectrofluorimeter (Ocean Optics, USA, 2010). Statistical processing of the results was carried out using the specialized software package OriginPro 7.5 (OriginLab Corporation, USA). The results were obtained in n-fold repetition. Also, the statistical reliability of the values between the experimental results and the control group was calculated based on Student's t-test and was assessed as statistically significant at values of $p<0.05$, $p<0.01$.

RESULTS OBTAINED AND THEIR ANALYSIS

In our experiments, the effect of Plantagin polyphenol on the NMDA receptor was studied using Zn2+ (5 µM), Mg2+ (5 µM) ions and glycine (50 µM). It was found that Ca2+ transport in synaptosomes remained virtually unchanged under the influence of Plantagin polyphenol under conditions of incubation of L-glutamate-Fluo-4 AM in a suspension of rat brain synaptosomes with Zn2+ and Mg2+ (5 µM). However, it was found that the activating effect of glycine was relatively suppressed in the presence of glycine (50 µM) (Figure 1).

The results of this experiment allow us to conclude that the polyphenol Plantagin used has both a stimulating and competitive/blocking effect on antagonists and agonists that directly affect the functional activity of the ionotropic NMDA receptor.

In experiments, glycine binds to the active site of the NMDA receptor, activating the calcium channel

through the receptor. In subsequent experiments, studies were conducted to determine the complex effect of 50 µM glycine incubated with Fluo-4AM in the presence of 50 µM concentration of the polyphenol Plantagin (Figure 1).

It turns out that glycine binds to the glycine-binding site of NMDA receptors, promoting activation of the Ca2+ channel. The obtained results show that the polyphenol Plantagin inhibits the increase in calcium dynamics caused by glycine.

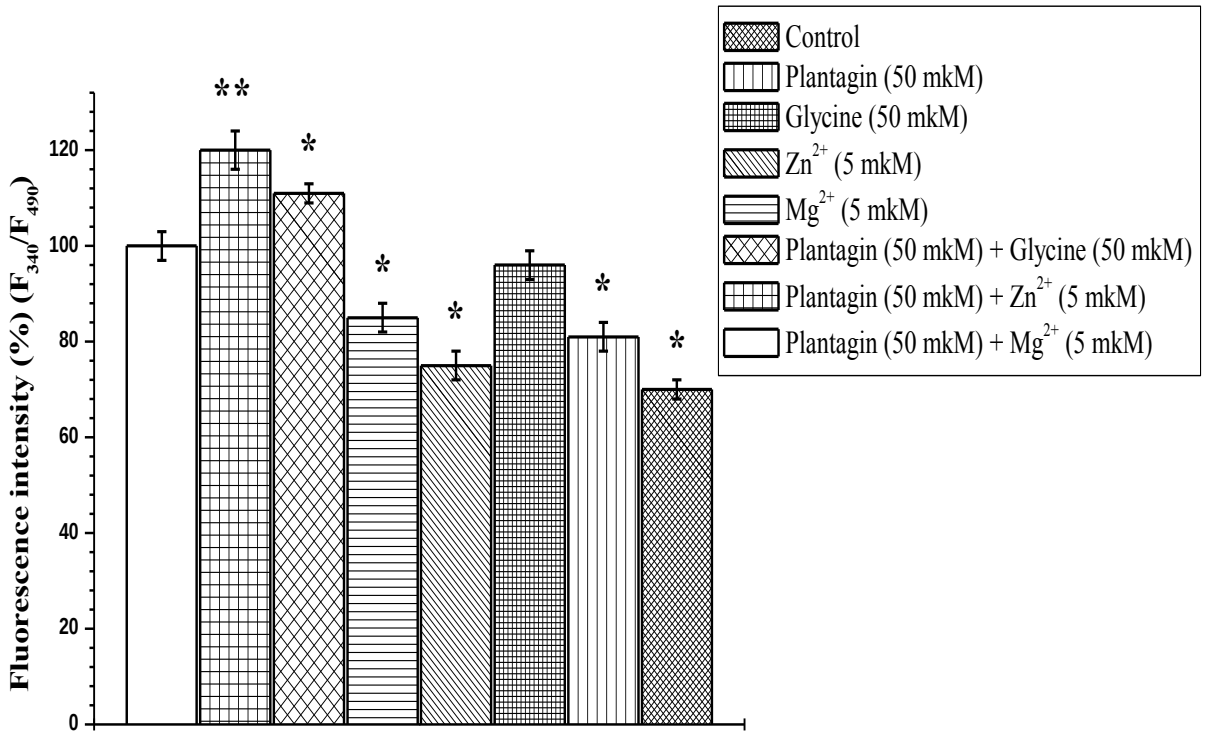

Figure 1. Effect of polyphenol plantagin (50 µM) on fluorescence intensity in synaptosomal suspensions of rat brain incubated with glycine (50 µM), Zn2+ (5 µM) and Mg^{2+} (5 µM). Confidence level. *- P<0.05; **- P<0.01.

This suggests that the polyphenol Plantagin may also affect glycine binding sites, suggesting that this polyphenol may be effective in receptor modulation.

In terms of discussion of the results, the polyphenol Plantagin competes with glycine for binding to the glycine site of NMDA receptors, reducing the likelihood of full receptor activation even in the presence of glutamate. This partial inhibition results in a limitation of Ca2+ influx and is therefore important for preventing excitotoxicity. Competition with glycine reduces NMDA receptor activation and the subsequent increase in calcium influx.

Normally, activation of NMDA receptors opens an ion channel, allowing Ca2+ to enter the postsynaptic neuron. In neurodegenerative diseases, disruption of this process leads to excessive calcium influx, which can trigger a cascade of harmful processes, including mitochondrial dysfunction, generation of reactive oxygen species (ROS), and activation of enzymes that destroy calcium-dependent cellular structures. The polyphenol Plantagin inhibits the entry of Ca2+ into the postsynaptic neuron by blocking the glycine site and partially inhibiting the activation of the NMDA receptor. This process reduces intracellular calcium concentration and stabilizes calcium homeostasis, preventing the harmful effects of excess calcium.

Plantagin polyphenols reduce the harmful effects of receptor hyperactivation by competitively blocking postsynaptic NMDA receptors at the glycine site. This blockade limits calcium influx, reduces excitotoxicity and protects neurons from damage and death.

NMDA receptors are a subtype of glutamate receptors involved in synaptic plasticity and learning. NMDA receptor antagonists prevent calcium ions from entering the cell by blocking the NMDA receptor channel. This process helps regulate glutamate-mediated excitatory signaling and prevents excessive calcium influx that can lead to neuronal damage. Examples of NMDA receptor antagonists include ketamine, memantine, and dextromethorphan.

Memantine binds to NMDA receptors more efficiently than Mg+ ions. It counteracts the long-term influx of

Ca2+ ions through the NMDA calcium channel (which counteracts the voltage-dependent blockade of NMDA receptors by Mg+ ions due to excess glutamate levels) by temporarily maintaining the physiological activity of the channel due to the high concentration of glutamate released from the synaptic terminal. Control of intracellular Ca2+ concentration is critical for neurons because it determines their survival and physiological function. Because memantine is voltage dependent, it dissociates the average physiological signal from its context and mediates average signal transmission.

Based on the above data, in our experiments the polyphenol Plantagin was compared with the drug Memantine, a non-competitive NMDA receptor blocker. The effect of the polyphenol plantagin used in these studies on the activity of synaptosomal Ca2+ channel suspensions in the presence of memantine was investigated (Figure 2).

In our experiments, we found that psyllium polyphenols (50 µM) at memantine concentrations (50 µM) had little effect on the calcium content of synaptosome suspension compared to memantine, indicating that this polyphenol does not affect the memantine binding site.

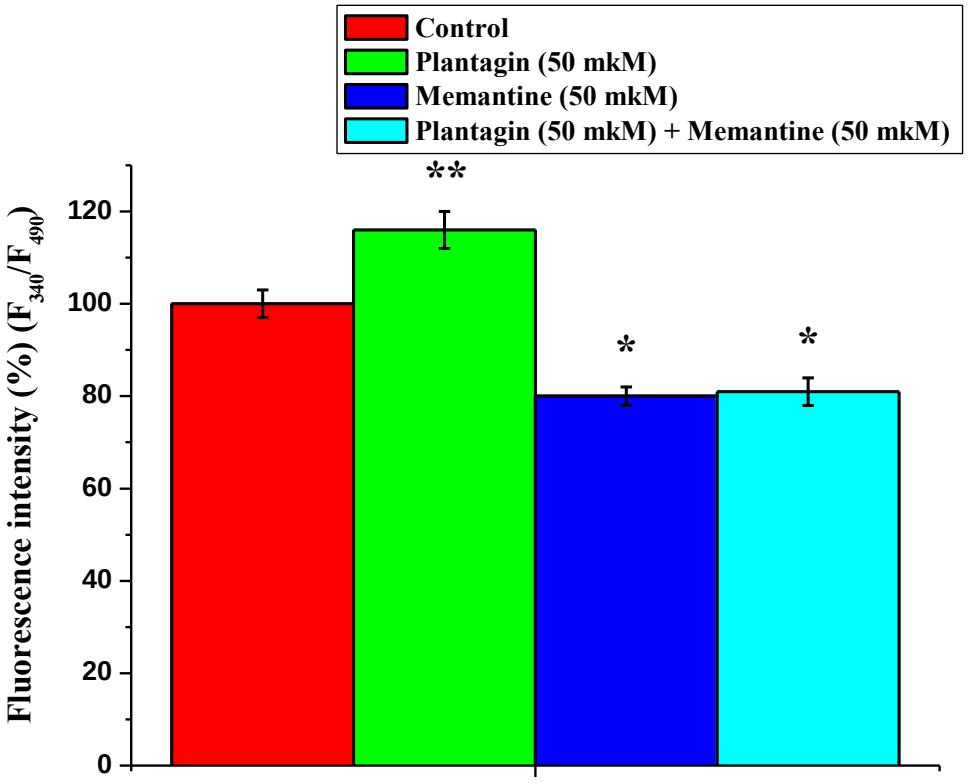

Figure 2. Effect of polyphenol plantagin and memantine (50 µM) on the fluorescence intensity of Fluo4-AM in synaptosomal suspensions of rat brain. Level of significance. *- P<0.05; **- P<0.01; *- P<0.001. (n=6).**

The results of the study indicate that the polyphenol Plantagin used does not affect the Mg2+ binding sites of the NMDA receptor.

CONCLUSIONS

It was found that synaptosomal Ca2+ transport was virtually unchanged in the presence of plantago polyphenols under Zn2+ and Mg2+ incubation conditions of L-glutamate-Fluo-4 AM in rat brain synaptosomal suspensions. The polyphenol Plantagin competes with glycine for binding to the glycine site of NMDA receptors, reducing the likelihood of full activation of the receptor even in the presence of glutamate. This partial inhibition results in a limitation of Ca2+ influx and is therefore important for preventing excitotoxicity. The results of this experiment allow us to conclude that the polyphenol Plantagin used has both a stimulating and competitive/blocking effect on antagonists and agonists that directly affect the functional activity of the ionotropic NMDA receptor.

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