EFFECT OF N-PHTHALAMOYL-L-GLUTAMIC ACID, A SELECTIVE NMDA RECEPTOR AGONIST, ON BINDING OF ^{3}H-L-GLUTAMATE WITH HIPPOCAMPAL SYNAPTIC MEMBRANES

O. N. Koreshonkov, P. V. Perestenko, M. A. Dumpis, and L. B. Piotrovskii

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The study of glutamatergic synaptic transmission has recently become one of the most rapidly developing trends in modern neurobiology. According to the existing classification, which is based mainly on the results of electrophysiological experiments, glutamate receptors can be subdivided into five main types: N-methyl-D-aspartate (NMDA), kainate, AMPA, AP4, and metabotropic [12].

Receptors of NMDA type have been most widely studied. This is because of their involvement in the mechanism of complex integrative processes such as learning, memory, etc. [5], and in the pathogenesis of several brain diseases (epilepsy, Aizheimer's disease, Huntington's chorea, ischemic neuronal degradation, etc.) [6, 9, 10, 13]. In connection with the facts described above, the study of substances capable of interacting with receptors of NMDA type and the creation of therapeutic substances on their basis are of great interest.

One such compound is N-phthalamoyl-L-glutamic acid (PhGA), a new selective agonist of NMDA receptors [8]. During the study of the action of PhGA on pyramidal neurons of the rat hippocampus it was found that: a) a response to the action of PhGA is exhibited only in the presence of glycine; b) the reversal potential of PhGA-induced currents is identical to that for aspartate-induced currents; c) the response evoked by PhGA is selectively blocked by Mg^{2+} ions, which are blockers of the ionic channels of NMDA receptors: competitive (AP5) and noncompetitive (kynurenate); d) complete cross-desensitization of responses to aspartate and PhGA is observed. However, after injection of PhGA into the cerebral ventricles of mice, it does not possess an excitatory action, but prevents seizures evoked by kainate and NMDA [1].

The aim of this investigation was to study the effect of PhGA on interaction between L-glutamic acid (L-Glu) and synaptic membranes of the human hippocampus, and to compare it with the action of NMDA.

EXPERIMENTAL METHOD

To obtain synaptic membranes (SM), autopsy specimens of the human hippocampus were used (autopsy was performed not later than 1-3 h after death, on persons not suffering from neurologic diseases). The material was homogenized in 10 volumes of 0.32 M sucrose, containing 0.1 mM phenylmethylsulfonyl fluoride and 0.1 mM EDTA. Synaptic membranes were isolated by differential centrifugation [15] and purified by exclusion chromatography on a column measuring 5 × 60 cm, packed with Sephadex G-25 ("Pharmacia," Sweden). Elution was carried out with buffer A: 50 mM Tris-HCl, pH 7.4.

To study binding of L-glutamate with synaptic membranes, 40-100 μg of membrane protein was incubated for 30 min at 25°C in 100 μl of a mixture containing 50 mM Tris-HCl, pH 7.4, and 3.9-800 mM ^{3}H-L-Glu. To study the effect of NMDA and PhGA on binding of L-glutamate, the substances were added to the incubation medium. The reaction was initiated by adding the membranes. Each point corresponded to four repetitions. Nonspecific binding

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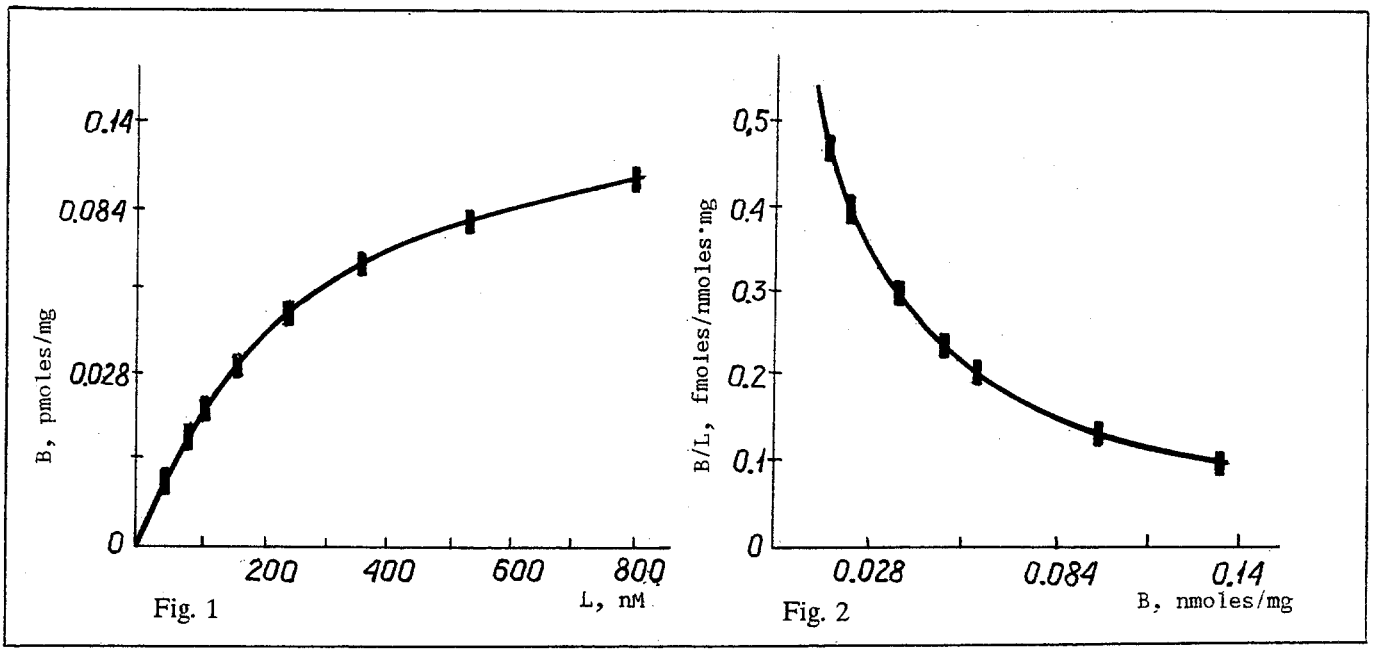

Fig. 1. Dependence of specific binding (B) of glutamic acid with synaptic membranes of the human hippocampus on concentration (L) of ^{3}H-L-glutamic acid.

Fig. 2. Scatchard plot of experiments to show specific binding (B) of labeled glutamic acid with human hippocampal synaptic membranes. L) Concentration of free ligand.

was determined in the presence of 1 μM unlabeled L-Glu in the incubation mixture. The unbound ligand was separated by filtration in vacuo through GF/C glass filters ("Whatman," England), treated with 0.3% polyethylenimine [4], with the aid of a 96-well "Minifold" ultrafiltration apparatus ("Schleicher and Schüll," Germany); the filters were washed with buffer A (5 × 250 μl), cooled to 0°C, after which they were dried and filled with 5 ml of scintillator based on toluene. The data were subjected to regression and difference methods of analysis of specific binding.

EXPERIMENTAL RESULTS

The results of the study of binding of ^{3}H-L-Glu with human hippocampal synaptic membranes are given in Figs. 1 and 2. The results of determination of the dependence of specific binding on concentration of the ligand suggest the existence of two binding sites with reception parameters of: K_{d1} = 0.35 ± 0.11 nM, B_{max1} = 6.5 ± 2.3 pmoles/mg, and K_{d2} = 51 ± 12 nM, B_{max2} = 98 ± 17 pmoles/mg respectively.

There is contradictory information in the current literature on the number of specific binding sites of ^{3}H-L-Glu with synaptic membranes. For instance, two binding sites, with values of K_{d1} = 11 nM and K_{d2} = 570 nM respectively have been found on synaptic membranes of the rat hippocampus [14], whereas in [3] only one binding site with K_d = 770 nM was found. A study of interaction of ^{3}H-L-Glu with SM in the rat cerebral cortex revealed two binding sites with K_{d1} = 60-70 nM and K_{d2} = 208-295 nM [11]. Differences in binding parameters are evidently linked with the conditions and techniques of the experiments and the method of analysis of the data. A study of the functioning of ionic channels of receptors of excitatory amino acids showed more than one binding site for L-Glu [7].

The value of the binding constant for the first site, which we determined, indicates the presence of a super-high-affinity binding site for glutamic acid. The presence of such a superhigh-affinity site was demonstrated previously for opiate receptors [2]. It has been possible to prove the existence of this binding site, in our view, first, through the introduction of a stage of chromatographic purification of SM, for it is possible, by exclusion chromatography, to remove low-molecular-weight components, including, perhaps, compounds affecting the binding of L-Glu, from the membrane fraction, and second, by the use of the difference method to analyze specific binding of the ligand.

Transformation of the data to Hill's coordinates gives the value of 0.23 for Hill's coefficient, evidence of negative cooperativeness in the system. The existence of such cooperative effects, hypothetically connected with allosteric regulation, in the system was noted in [7].

These investigations enabled the following values of inhibition constants of ^{3}H-L-Glu binding for NMDA and PhGA respectively: K_i(NMDA) = 19 μM and K_i(PhGA) = 13 μM. Addition of glycine, a selective modulator of NMDA receptors, to the incubation medium did not affect the parameters of L-Glu binding, but potentiated the inhibitory action of NMDA and PhGA.

These results confirm yet again the conclusion that PhGA is an agonist of NMDA receptors [8]. However, it is clear from the results obtained on isolated neurons that activity of PhGA is 2 orders of magnitude less than that of NMDA. This may perhaps explain the ability of PhGA to block NMDA-induced seizures [1]. Although it has virtually the same affinity for the receptor as NMDA, PhGA occupies the binding sites and does not allow NMDA molecules to interact with them and induce seizures. The absence of an excitatory effect of PhGA itself can be explained by its low "inner activity." Consequently, PhGA is a partial agonist of glutamate receptors of NMDA type.

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