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The neuropeptide cycloprolylglycine can form a complex with AMPA receptors

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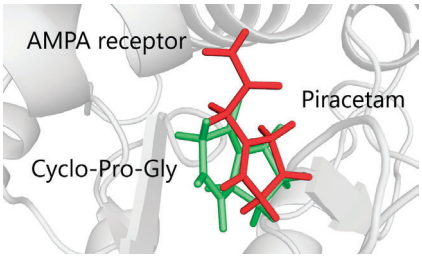
The calculations using the MM-GBSA method demonstrated that the neuropeptide cycloprolylglycine, structurally and pharmacologically resembling the classical nootropic piracetam, can bind to the piracetam site of the ligand-binding domain of the GluA3i AMPA receptor subtype with the binding energy higher than that of piracetam. Thus, together with the previous electrophysiological and biochemical data, the computational results confirm that cycloprolylglycine may be an endogenous ampakine.



Keywords: allosteric site of AMPA receptor, endogenous ampakine, cycloprolylglycine, piracetam, MM-GBSA.

Cycloprolylglycine (CPG), the endogenous cyclodipeptide identified in rat brain,[1] human blood plasma and cerebrospinal fluid,[2] was initially proposed as a topological peptide analog of the classical nootropic piracetam (*N*-carbamidomethylpyrrolidone-2). CPG not only has the similar structure (Figure 1), but also possesses the main pharmacological activities of piracetam, including nootropic,[3] antihypoxic,[4] neuroprotective and anxiolytic[5,6] in 100–1000-fold lower doses.

Previously, the researchers of the V. V. Zakusov Research Institute of Pharmacology hypothesized that CPG can be an endogenous positive modulator of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, based on electrophysiological experiments[7] and pharmacological inhibitory analysis data.[8]

This hypothesis is consistent with the fact that piracetam, like other racetams, implements its effects through the positive modulation of AMPA receptors, as has been demonstrated back in the 90s.[9] Not so long time ago, the allosteric binding sites of piracetam and aniracetam were identified on AMPA receptors by X-ray diffraction analysis.[10]

In addition to racetams, other synthetic compounds interact with allosteric binding sites of AMPA receptors, for example, cyclothiazides (CX614, CX546, PEPA, IDRA-21),[11] isobutyric and cycloalkanecarboxylic acids derivatives with 1,4-bis(aminomethyl)benzene linker[12,13] and a lot of other compounds with different binding sites. The ways of binding

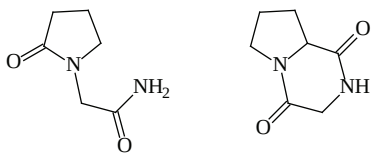


Figure 1 Structures of piracetam and CPG.

them may also vary significantly. All these exogenous compounds are called ampakines. The endogenous ligands of allosteric sites of AMPA receptors are unknown.

The aim of this work was to obtain additional evidence for the hypothesis that CPG is an endogenous ligand of AMPA receptors.

For this purpose, piracetam was replaced with CPG in the crystal structure of the complex of piracetam with extracellular agonist-binding S1S2 domain GluA3i of the AMPA receptor subtype (PDB ID: 3LSX), then the binding energies of piracetam and CPG to AMPA receptor were calculated by molecular mechanics using the generalized Born model and taking into account the available molecular surface area (MM-GBSA).[14,15]

The coordinates of the atoms of a piracetam molecule were obtained from the spatial structure of the PDB ID: 3LSX. The CPG particulars were taken from the PDB (Ligand ID: GIO) The CPG molecule was placed in the piracetam binding site using the Coot0.9.6 program.[16] The software packages AmberTools18 and Amber18 were used for modeling.[17] At first, the topologies of the receptor, its complexes with CPG and piracetam, and separately the ligand molecules were modeled. The antechamber program was used to parametrize the ligands.[18] Water and ions were added to the complexes to neutralize the charges of the systems. The AMBER14SB[19] field was used as a force field.

The balancing of each receptor–ligand system was carried out in four stages: at first, a short energy minimization, followed by heating at 300 K for 50 ps. Then the density of the systems was balanced with restrictions on the atom mobility for 50 ps followed by balancing for 500 ps at a pressure of 1 atm using a Langevin thermostat[20] and a Berendsen barostat[21] with 2 fs time step with the restrictions on the atom mobility removed. Long-range electrostatic interactions were calculated using the Ewald summation scheme.[22] The balancing of the system was considered complete if the cessation of significant changes in the density, temperature, and total energy of the system was observed.

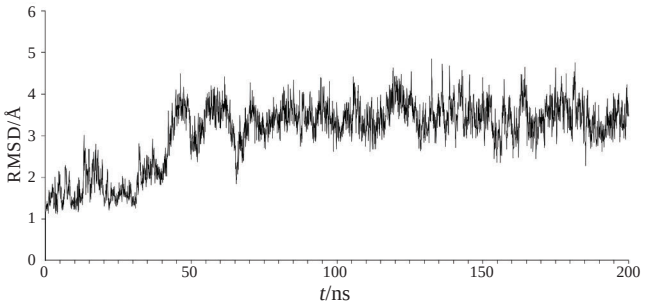


Figure 2 Change in RMSD of the atom positions of the CPG–AMPA receptor complex.

The root mean square deviations (RMSD) of the atom positions in the receptor–CPG systems are shown in Figure 2.

The molecular dynamics were modeled over a period of 200 ns. The coordinates of the atoms were recorded every 100 ps, and as a result, a set of 2000 frames was obtained for each of the complexes.

To calculate the free energy of the system, the MM-PBSA.py program was used.[23] A modified generalized Born model was used, which is intended for macromolecules.[24]According to the calculations, CPG can be situated in the same site of the extracellular S1S2 domain of the GluA3i subtype of the AMPA receptor as piracetam (Figure 3).

As a result of the calculations, the values of the free energy change during the binding of piracetam and CPG to the S1S2 domain of the flip form GluA3i of the AMPA receptor subtype were obtained (Table 1).

CPG binds to the receptor stronger than piracetam, which is indirectly confirmed by the values of their pharmacologically effective doses. Of course, this comparison is only an estimation due to the impossibility of direct comparison of binding energies obtained by MM-GBSA calculations and experimentally determined bioactivity values (especially *in vivo*). The weak binding of piracetam to GluA3i is consistent with the relatively low occupancy of this binding site by piracetam according to the X-ray diffraction analysis.[11] The nearest calculated CPG environment in the allosteric site of the AMPA receptor is represented by the same residues as the nearest piracetam environment according to the X-ray diffraction data (Figure 2). It includes five hydrophilic residues (Asn252, Asp248, Ser217, Asp216 and Asn214) and one hydrophobic (Leu215). CPG has greater affinity to the AMPA receptor probably due to the interaction between Asn252 and Gly moiety of CPG, which is absent in the case of piracetam, and the interaction between carbonyl group of CPG and Asp 248 manifested by the distance of 3.87 Å compared with 3.96 Å between Asp 248 and amide group of piracetam.

Table 1 The free energy changes associated with piracetam and CPG binding by the AMPA receptor.

Ligand	Calculated value of the free energy change during ligand binding to GluA3i/ kcal mol^{-1}	Optimal effective dose of CPG and piracetam intraperitoneally/mg kg^{-1}	
		antihypoxic[4] (mice)	nootropic[3] (rats)
Piracetam	−2.37 ± 0.03	900	200
CPG	−3.99 ± 0.04	1.0	0.1

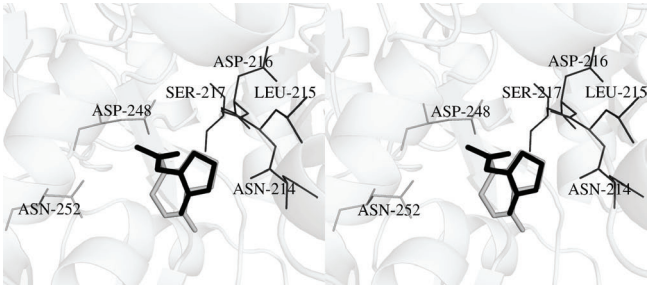


Figure 3 The stereoview of CPG (light) and piracetam (dark) locations in the allosteric binding site of the GluA3i subtype of the AMPA receptor after balancing the systems. The amino acid residues of the receptor within a circle with the radius of 4 Å around each of the ligands are represented by the lines.

Thus, the results of the molecular dynamics modeling demonstrated that CPG can form a complex with the GluA3i subtype of AMPA receptors like piracetam, but with greater affinity. These data suggest that CPG can be an endogenous ampakine.

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