Biological Science

NE3A, NW12A, 5A, 17A/2009G501

Structure-based drug design for kainate-type ionotropic glutamate receptors, GluK1 and GluK2, from human brain

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Introduction

Glutamate receptors are located on neuron membranes and play a central role, not only in excitatory neurotransmission but also in complex brain functions such as learning and memory and are thought to be involved in several neurological disorders. Ionotropic glutamate receptor (iGluR) subunits constitute a large family of ligand-gated ion channels responsible for the majority of excitatory synaptic transmission by mediating influx of cations into the post-synaptic cell. This gene family is divided into *N*-methyl-D-aspartate (NMDA) and non-NMDA receptor subunits. The non-NMDA iGluRs are further subdivided into α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate receptor subunits. Molecular cloning studies demonstrated that non-NMDA iGluRs are encoded by at least four AMPA (GluA1-4), and five kainate receptor genes (GluK1-5). The iGluRs are integral membrane proteins assembled from subtype specific sets of homologous subunits into homo- and heteromeric complexes with distinct functional properties. Understanding the complex roles of iGluRs has been facilitated by the presence of selective pharmacological agents. However, pharmacological characterization of kainate receptors has for many years been hampered by the lack of selective ligands.

Dysiherbaine (DH), isolated from the Micronesian sponge *Lendenfeldia chondrodes* is the high-affinity agonists of GluK1 and 2, subtypes of the kainate receptors. The molecular structure of DH consists of a *cis*-fused hexahydrofuro[3,2-*b*]pyran ring system containing a glutamic acid substructure. Neodysiherbaine A (NDH), isolated as a minor congener from the same sponge, differs from DH in the C_8 functional group. NDH is similar to DH in its pharmacological activity on kainate receptors, albeit with slightly different binding affinity. It is important to understand the interactions that occur in the ligand-binding domain to understand basis for the selective binding of the ligands.

What nobody understands is how some DH analogues discriminates the quite similar isoforms, GluK1 and 2. To try to get at the question, we have tried solving 3D structures of human GluK1 and 2 in complexes with the DH analogues at high resolution. It is a benefit to discuss the small structural changes in binding these ligands. Due to their unique structures of DH and NDH, these structures would serve useful information to design the novel subtype-selective drugs. Also, they will be used as the tools for further understanding the structures and functions of iGluRs.

Experiments

Expression of the ligand-binding cores of GluK1 and 2

The subunit of iGluR contains a ligand-binding core, which consists of two sub-domains, namely S1 and S2 domains. The construct for expression of the ligand-binding core of human GluR5 (hGluR5) was made as follows (1). The S1 and S2 segments constituting the ligand-binding core were polymerase chain reaction (PCR)-amplified from cDNA. PCR-products were digested with restriction enzymes, and the digested inserts were ligated into pCold (Takara) and pET-28a(+) (Novagen) for GluK1 and 2, respectively, in a three-point ligation. The expressed proteins were connected by a Gly-Thr linker. The ligand-binding cores were co-expressed with GroEL/ES using BL21(DE3) transformed by chaperon plasmid pGro7. A higher yield of active protein could be achieved after cell harvesting and centrifugation.

Crystallization and X-ray diffraction experiments

The crystallization conditions were searched based on the previously reported conditions in which the glutamate was bound to the ligand-binding core (1). For NDH-bound GluK2 crystals, oxidized and reduced forms of glutathione were added as the additive regents.

X-ray intensity data collection experiments were conducted at Photon Factory (Tsukuba) and SPring-8 (Harima). For GluK1, the complexes with DH, NDH, 8-epi-NDH, 8-deoxy-NDH, 9-deoxy-NDH and 8, 9-dideoxy-NDH (MSVIII-19) were co-crystallized and all the intensity data were obtained at 1.5 Å resolution. For GluK2, the complexes with DH and NDH were co-crystallized and the data were obtained at 2.05 and 1.65 Å resolution, respectively.

Structures were successfully solved by molecular replacement method and small structural differences were analyzed.

Some interesting features for the DH analogues in complexes with GluK1 and 2 will be reported, soon.

References

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