Biological Science

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

Discrimination of kainate-type ionotropic glutamate receptors, GluK1 and GluK2, by the toxins, neodysiherbaine A and its analogues

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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 (KAR) 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) and neodysiherbaine A (NDH), isolated by Sakai and coworkers from the marine sponge *Lendenfeldia chondrodes*, are potent agonists that selectively bind to the GluK1 and GluK2 KAR isoforms. Structurally, DH and NDH consist of a *cis*-fused hexahydrofuro[3,2-b]pyran ring system with two functional groups at the C_8 and C_9 positions, in addition to a glutamate substructure. Because of a difference in the functional groups at C_8 (*N*-methylamino group in DH and hydroxy group in NDH), the affinity of DH for GluK1 is 15-fold higher than that of NDH. Characterization of the synthetic NDH analogues revealed that 8-deoxy-NDH, which lacks the C_8 hydroxy group of NDH, binds to GluK1 as strong as NDH does, but 9-deoxy-NDH and 8,9-dideoxy-NDH (MSVIII-19), which lack the C_9 and C_8/C_9 functional groups, respectively, has the lower affinities than NDH. DH and NDH also strongly bind to GluK2, but 8-deoxy-NDH has much lower affinity for GluK2 than those of DH and NDH, and 9-deoxy-NDH and MSVIII-19 do not bind to GluK2. This is unexpected, because these compounds have quite similar chemical structures, and because GluK1 and GluK2 have a very high sequence identity (74 % in overall, 87 % in ligand-binding core). To solve this mystery, it is important to know the binding modes of DH, NDH and their analogues to KARs at atomic level. Knowing the binding modes is also useful to rationally develop KAR isoform selective compounds those is useful for understanding of the exact functional and pathological roles of distinct KARs.

Results

Determined structures

We have determined crystal structures of the human GluK1 ligand-binding core (hGluK1-S1S2) in complexes with DH, NDH, MSVIII-19, 8-deoxy-NDH, and 9-deoxy-NDH at 1.5 Å resolution, along with the L-glutamate-complex at 1.65 Å resolution. These ligands similarly bind to hGluK1-S1S2 by many hydrogen-bonding interactions and some van der Walls interactions, but the C_9 functional group deletion causes the conformational changes of the ligand to the flattened or twisted conformations by losing the intramolecular hydrogen bonds. The conformational changes not only decrease number of the hydrogen-bonding interactions but also generate steric hindrance with hGluK1-S1S2 protein matrix. Furthermore, we have determined the crystal structure of human GluK2 ligand-binding core (hGluK2-S1S2) in complex with NDH, as well. NDH binds to GluK2 in a manner different from that seen in GluK1 due to the differences in three amino acid residues interacting with the ligand. This difference, in addition to the conformational changes of the ligands, is likely to make the selectivity of 8-deoxy-NDH, 9-deoxy-NDH, and MSVIII-19 between GluK1 and Gluk2.

Table 1: Crystallographic statistics

complex	GluK1 L-glutamate	GluK1 DH	GluK1 NDH
R/R_{free} (%)	19.4/22.5	19.9/22.5	19.9/23.5
GluK1 MSVIII-19	GluK1 8-deoxy-NDH	GluK1 9-deoxy-NDH	GluK2 NDH
16.8/19.2	16.6/19.2	17.1/18.9	16.5/20.5

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