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Binding and Selectivity of the Marine Toxin Neodysiherbaine A, and its Synthetic Analogues, to GluK1 and GluK2 Kainate Receptors

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1 Introduction

Ionotropic glutamate receptors (iGluRs) are synaptic receptors that form L-glutamate-gated ion channels and play central roles in excitatory neurotransmission in the mammalian central nervous system. Pharmacologically, iGluRs are classified into three broad subfamilies: NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate), and kainate receptors (KARs). The iGluR subfamily is comprised of a total of eighteen proteins. Isoforms within each subfamily assemble into homo- or heteromeric oligomers in certain combination in order to form functional ion channels. Thus, functional synaptic receptors are highly diverse and inherently difficult to characterize.

Dysiherbaine (DH) and neodysiherbaine A (NDH), isolated from the marine sponge *Lendenfeldia chondrodes*, provide a particularly interesting example of such a molecule. DH and NDH have been shown to be potent convulsants in mice and have been characterized as potent agonists for KARs. DH has also been shown to bind selectively and to have unusually high affinity for both GluK1 (formerly known as GluR5) and GluK2 (GluR6) KAR isoforms, and can selectively activate subunits in some heteromeric receptor complexes. Thus, DH, NDH and their analogues may serve as interesting tools which characterize neuronal KARs in detail.

Structurally, DH and NDH contain a shared template, consisting 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. Since relatively small differences in the functional groups at C_8 were found to impart significant effects upon binding affinity and selectivity, as revealed by comparing the activity of DH and NDH, a series of NDH analogues including 8-deoxy-NDH, 9-deoxy-NDH, and 8,9-dideoxy-NDH (MSVIII-19), were synthesized. DH and NDH also bind strongly to GluK2, although 8-deoxy-NDH has a much lower affinity for GluK2 than those of DH and NDH. Furthermore, 9-deoxy-NDH and MSVIII-19 do not bind to GluK2. These binding profiles are rather unexpected considering the structural similarity of both ligands and receptors, where GluK1 and GluK2 have 74 % and 87 % identity overall in terms of ligand-binding core sequence identity, respectively. It has been shown that DH, NDH and 8-deoxy-NDH are full or efficient agonists for GluK1, while 9-deoxy-NDH is only a partial agonist. Most interest is that MSVIII-19 acts as a potential antagonist for GluK1. Furthermore, MSVIII-19 induced a coma-like sleeping state in mice when administrated intracerebroventricularly, rather than induce convulsions. Previously antagonists have been proposed to stabilize an "open" rather than "closed" state of the receptor-ligand complex. These drastic changes in the mode of activity and isoform selectivity, as a result of only a relatively small change in the functional group observed in NDH analogues, are intriguing issues to explore further.

2 Experiment

See PF Activity Report 2009 #27 Part B or [1].

3 Results and Discussion

We determined the structure of the human GluK1-S1S2 (hGluK1-S1S2) in complex with DH, NDH, MSVIII-19, 8-deoxy-NDH, and 9-deoxy-NDH, and obtained the structures at 1.5 Å resolution. In addition, we also obtained the structure of hGluK1-S1S2 complex with L-glutamate at 1.65 Å resolution. Finally, we determined the crystal structure of the human GluK2-S1S2 (hGluK2-S1S2) in complex with NDH. We found that differences in three amino acids (Thr503, Ser706, and Ser726 in GluK1 and Ala487, Asn690, and Thr710 in GluK2) in the ligand-binding pocket generate differences in the binding modes of NDH to GluK1 and GluK2. Furthermore, deletion of the C_9 hydroxy group in NDH alters the ligand conformation such that it is no longer suited for binding to the GluK1 ligand-binding pocket. In GluK2, NDH pushes and rotates the side chain of Asn690 (substituted for Ser706 in GluK1), and disrupts an interdomain hydrogen bond with Glu409. The present data supports the idea that receptor selectivities of DH analogues resulted from the differences in the binding modes of the ligands in GluK1/2 and the steric repulsion of Asn690 in GluK2. All ligands, regardless of agonist efficacy, induced full domain closure. Consequently, ligand efficacy and domain closure did not directly coincide with DH analogues and the kainate receptors.

References

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