Lead Author and Affiliation
Theanne Nicole Griffith

Author's School/Institution
Northwestern University, Chicago, Illinois

Academic Background: Date of Entry: September 2010
Degrees Expected: PhD
Expected Year of Graduation/Program Completion: 2015
Major or Field of Study: Neuroscience
Academic Level: PhD Candidate

Institution where the research was conducted
Northwestern University, Chicago, Illinois

Co-author(s)
Bryan A. Copits, PhD, Geoffrey T. Swanson, PhD

Co-authors' Affiliations
Northwestern University, Chicago, Illinois

Title: "Structural Determinants Responsible for the Role of Neto Proteins as Kainate Receptor Auxiliary Subunits"

Category: Cellular Biology, Neuroscience, Biochemistry or Physiology

Funding Source(s): *National Institute of Neurological Disorders and Stroke (NINDS) Grant (RO1 NS0719520) to G.T.S, Neurobiology of Information Storage Training Grant (5T32MH067564-09)*

Statement of the Problem/Background:
Kainate receptors (KARs, GluK1-5) are part of the ionotropic glutamate receptor family and play an important role in the modulation of synaptic transmission. KARs have been shown to complex with the auxiliary subunits Neto1 and Neto2. Neto proteins are single-pass transmembrane proteins containing two extracellular CUB domains, an LDLa module, and an intracellular C-terminus. Despite the gross structural similarities between these two proteins, they have been shown to have divergent effects on KAR biophysical function. We have previously shown that co-assembly of Neto1 with GluK1 homomeric receptors speeds desensitization kinetics while co-assembly with Neto2 slows receptor desensitization kinetics. Additionally, Neto2 increases the peak amplitude of GluK1 currents while Neto1 has no effect. Despite their important roles as KAR auxiliary subunits, the structural determinants that confer functionality to these closely related proteins remain unknown.

Research Question/Hypothesis:
In the present study, we examined the structural determinants present in Neto proteins responsible for their modulation of KAR desensitization kinetics. Specifically, we investigated the role of the CUB domains in Neto function. We hypothesized that these extracellular CUB domains are important for Neto modulation of KAR desensitization kinetics.

Research Design/Methods Used in the Investigation:
We co-expressed GluK1, GluK2 or GluK2/5 receptors in HEK293 cells with either Neto1 or Neto2 lacking the first or second CUB domain. Using voltage-clamp techniques, we fast-applied either glutamate or kainate to lifted HEK293 cells and measured KAR desensitization kinetics.

Results/Summary of the Investigation:
We found that loss of either CUB domain from Neto1 or Neto2 produced subunit and agonist specific effects. For example, loss of either CUB domain from Neto1 did not have a profound effect on glutamate-evoked current desensitization for either GluK1 or GluK2 homomeric receptors, whereas kainate-evoked currents were altered. On the other hand, loss of either CUB domain from Neto2 altered glutamate-evoked GluK1 and GluK2 receptor kinetics. Kainate was not tested with the Neto2 CUB-deletion mutants. Finally, a tail current is normally produced after fast application of glutamate when GluK2/5 receptors co-assemble with Neto1. However, a tail current was not observed when these receptors were expressed with Neto1 lacking the first CUB domain. This indicates that for these receptors, Neto1 is either altering agonist affinity or receptor entry into desensitization.

Interpretation/Conclusion of the Investigation:
In conclusion, the CUB domains of Neto proteins are important for their modulation of KAR desensitization kinetics. Furthermore, this modulation is KAR subunit and agonist specific, highlighting the functional diversity KAR co-assembly with different Neto proteins can provide.