FUNCTIONAL IMPACT OF AUXILIARY PROTEINS ON KAINATE RECEPTORS

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Summary

Fast excitatory synaptic transmission is mainly mediated by glutamate receptors in the Central Nervous System (CNS). This family of receptors comprises three different subfamily named after ligand preference: a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate. Among these, kainate receptors (KARs) are the less understood from a physiological point of view. Indeed, there is a mismatch in their functional properties when native receptors are compared with those expressed in heterologous systems. Recently, two auxiliary proteins for KARs, Neto1 and Neto2 (NEuropilin TOlloid-like 1 and 2) have been identified (1).

To assess the effect of these auxiliary proteins on the functional properties of KARs, we have coexpresed these with different subunits in HEK 293 cells. KAR-mediated responses were indiced by rapid agonist application and recorded with the voltage clamp technique under whole cell configuration. Current amplitude, desensitization rate and recovery of receptor desensitization, as well as agonist affinity were determined.

Our results show that Neto1 increases current amplitude of GluK2 and GluK3, speeds up recovery from desensitization and increases agonist affinity of the three major KARs subunits (GluK1, GluK2 and GluK3). Neto1 also altered desensitization onset, increasing GluK1 and GluK3, and reducing GluK2 desentization rate. Neto2 dramatically increased current amplitude, augmented agonist affinity and slowed desensitization rate of the three subunits, having a major action on GluK1. On the other hand, the recovery from desensitization remained unchanged when receptors were associated with Neto2.

According to these data, we conclude that Neto1 and Neto2 functionally interact with and modulate the three main KAR subunits but the degree and sign of modulation depend on the type of receptor. The existence of these interactions adds complexity to KARs functionality.

1. Zhang, W. et al., **Neuron** 61, 385-396 (2009)

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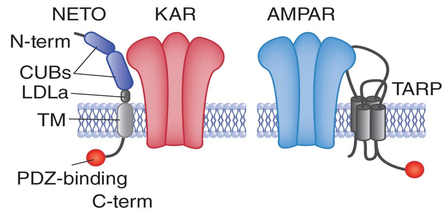
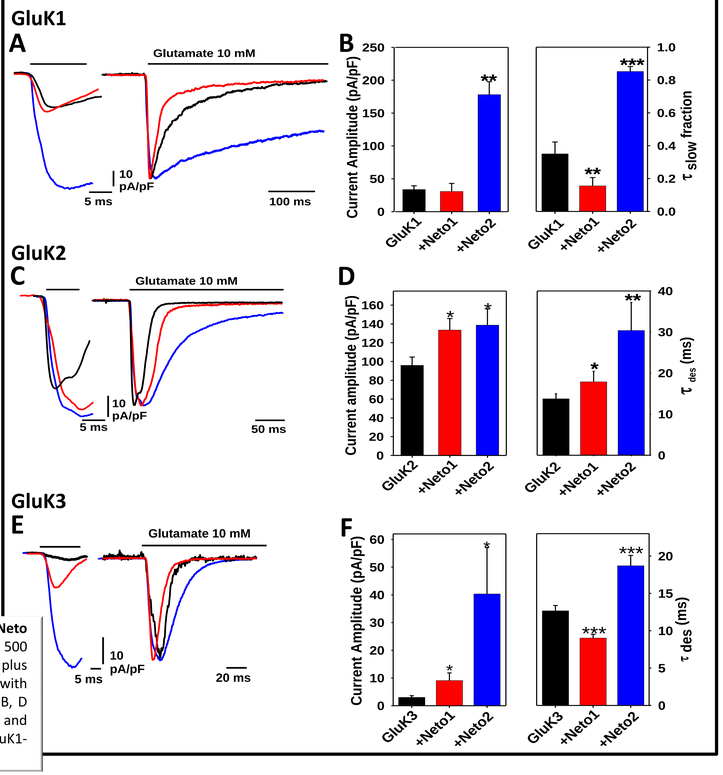
Figure 1. Schematic structure of KARs and Neto proteins



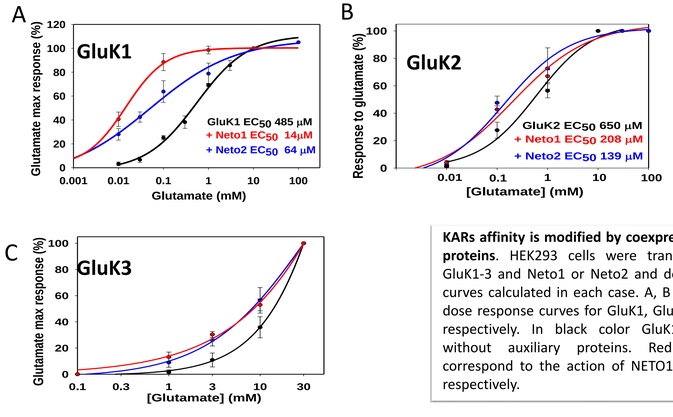
Structure of the known accessory subunits of glutamate-gated receptor channels, AMPA receptors and kainate receptors. The kainate receptor (KAR) auxiliary subunits Neto1 and Neto2 both contain two CUB domains and one LDLa domain that comprise the N-terminal (N-term) extracellular region. Each has one transmembrane (TM) domain, but they differ in the intracellular region, where only Neto1 has a PDZ-binding domain at the C terminus (C-term). The AMPA receptor (AMPAR) auxiliary subunits, TARPs, contain four transmembrane domains, with both the N and C termini located intracellularly.

Figure 2. Response Amplitude and Desensitization



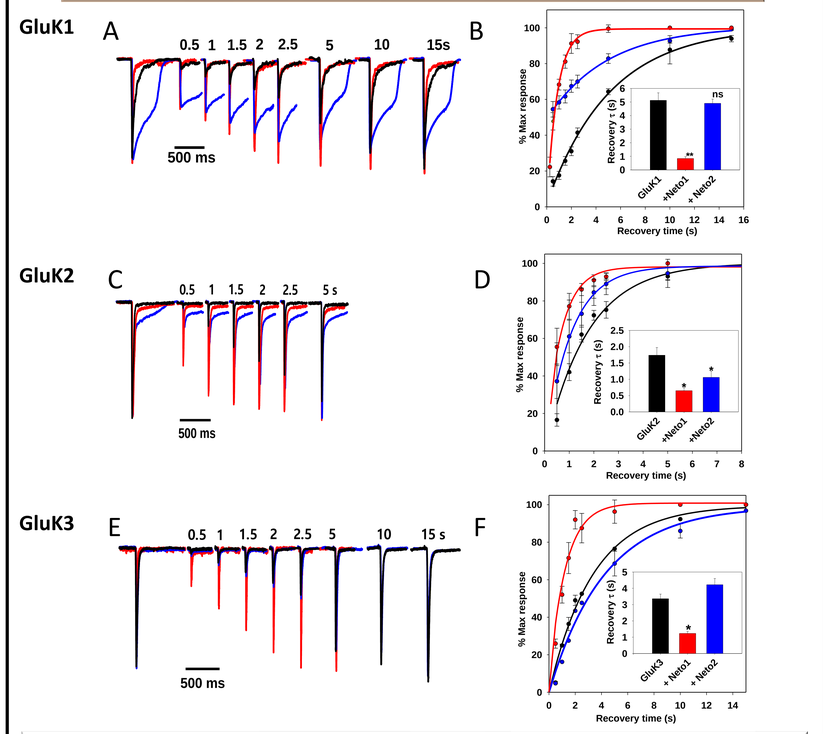
KARs amplitude and desensitization rate modification by Neto auxiliary subunits. Responses elicited by glutamate (10 mM, 500 ms) in HEK 293 cells transfected with different KARs subunit plus Neto1 or Neto2. Traces in A, C and E correspond to GluK1-3 with and without Neto proteins., before and after normalization. B, D and F represent quantification of current amplitude and desensitization rate. In black, KARs subunit GluK1-3; in red, GluK1-3 & Neto1 and in blue, GluK1-3 & Neto2.

Figure 3. Agonist affinity



KARs affinity is modified by coexpresion of Neto proteins. HEK293 cells were transfected with GluK1-3 and Neto1 or Neto2 and dose response curves calculated in each case. A, B and C show dose response curves for GluK1, GluK2 ad GluK3, respectively. In black color GluK1-3 receptor without auxiliary proteins. Red and blue correspond to the action of NETO1 and NETO2, respectively.

Figure 4. Recovery from desensitization



Vaiable effect of Neto proteins in KARs recovery rate. Recovery from desensitization was measured in HEK 293 cells transfected with KAR plus Neto proteins. KARs complexes were activated by 10 mM and 500 ms pulse of glutamate at different intervals. In A, C and E traces from GluK1-3 KAR (black) superimposed with reponses obtained when coexpresing Neto1 or Neto2 (red and blue, respectively). In B, D and F are plots measuring kinetics of recovery from desensitization, fitted to exponentials to measure the time constant (τ).

Data summary

Effect of Neto1 and Neto2 on properties of responses mediated by different KARs subunits (% of pure receptors)

	Amplitude		τ desensitization		Recovery rate		Affinity	
	Neto1	Neto2	Neto1	Neto2	Neto1	Neto2	Neto1	Neto2
GluK1	96%	573%	43%	242%	625%	95%	2898%	176%
GluK2	141%	146%	125%	213%	370%	83%	214%	286%
GluK3	303%	1348%	71%	148%	270%	89%	↑	↑