Functional Impact of Auxiliary Proteins on Kainate receptors

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Summary

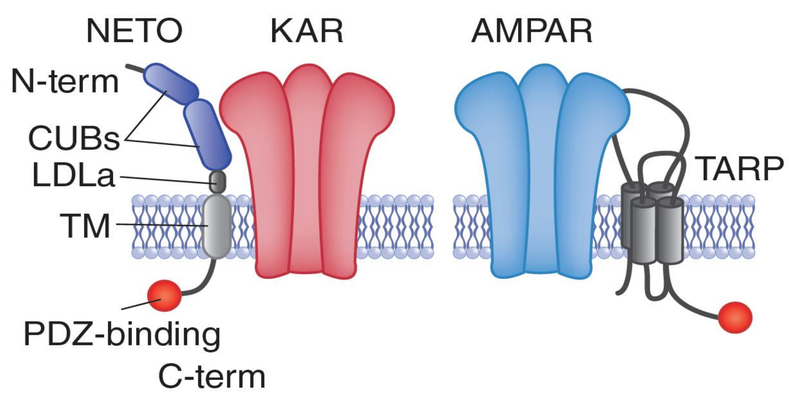
Fast excitatory synaptic transmission is mainly mediated by glutamate receptors in the Central Nervous System (CNS). This family of receptors comprises three different subfamily named after ligand preference: a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) 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 .

We tested the time expression profile of Neto1 and Neto2 in whole brain using RT-PCR to unveil differences between these proteins. To assess the effect of these auxiliary proteins on the functional properties of KARs, we have coexpresed Neto1 and Neto2 with different subunits of KARs in HEK 293 cells. KAR-mediated responses were indiced by rapid agonist application and recorded with the voltage clamp technique under whole cell configuration. Different biophysical properties such as, current amplitude, onset and recovery of receptor desensitization, agonist affinity and sodium dependence activation were determined.

Our results show a different developmental expression for Neto1 and Neto2. Functionally, Neto1 increases current amplitude of GluK2 and GluK3, and 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. Neto1 does not change GluK1 sodium dependence for channel gating, while it is reduced for GluK2. Neto2 dramatically increased current amplitude, augmented agonist affinity and slowed desensitization onset rate o 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. Sodium dependence is almost abolished for GluK1 Neto2 heteromers, while for GluK2 containing Neto2 complexes sodium dependence is reduced but still present.

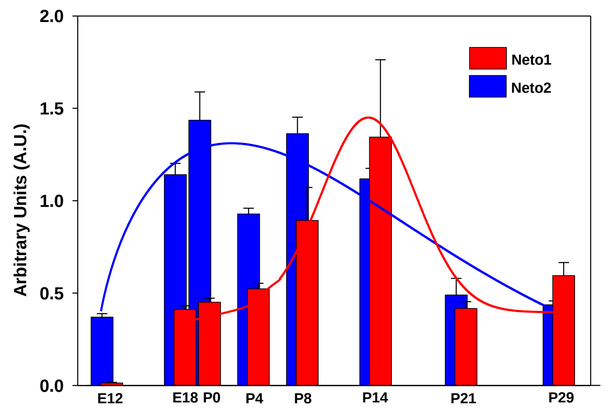
According to these data, we conclude that Neto1 and Neto2 functionally interact with and modulate the three main KAR subunits but the degree and sign of modulation depend on the type of receptor. The existence of these interactions adds complexity to KARs functionality.

Figure 1. Schematic structure of KARs and Neto proteins



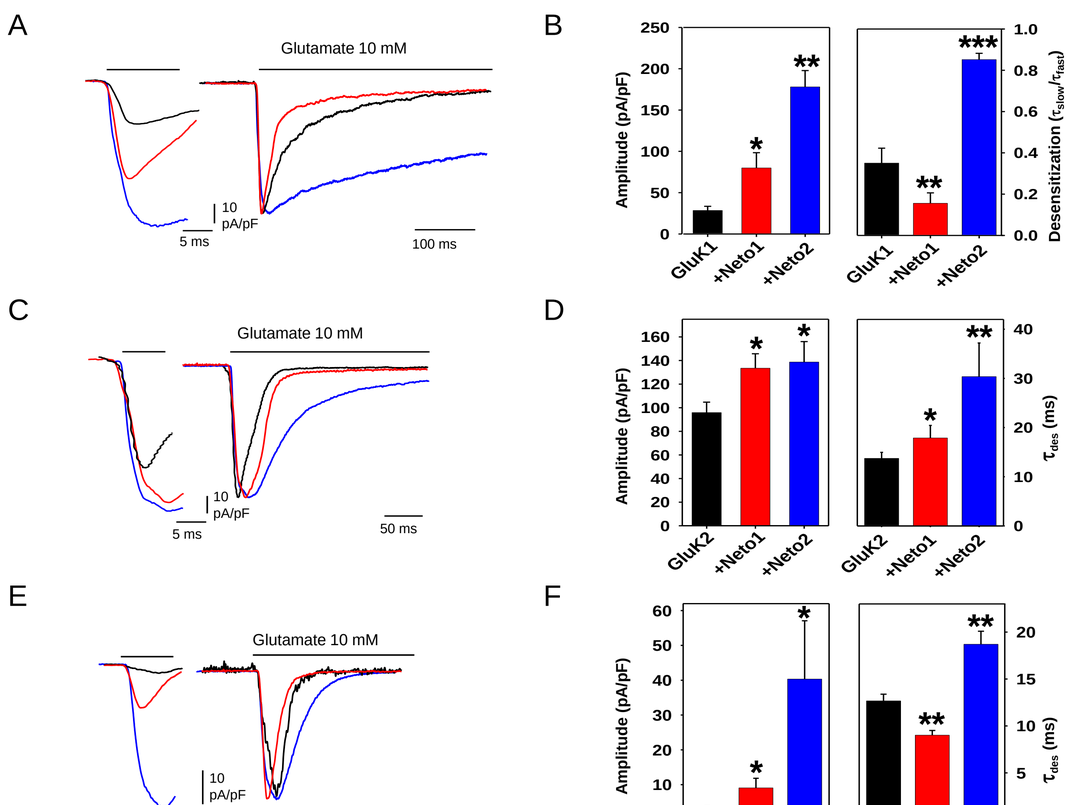
Structure of the known accessory subunits of glutamate-gated receptor channels, AMPA receptors and kainate receptors. The kainate receptor (KAR) auxiliary subunits Neto1 and Neto2 both contain two CUB domains and one LDLa domain that comprise the N-terminal (N-term) extracellular region. Each has one 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. Neto1 and Neto2 gene expresion during development



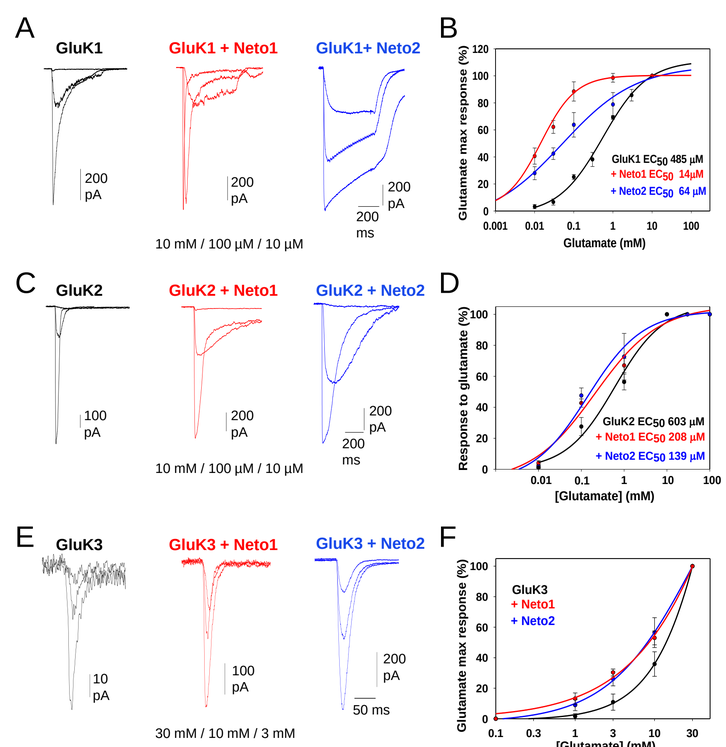
Neto1 and Neto2 gene expression levels during development in mice. Quantitative PCR analysis of Neto1 and Neto2 mRNA at different key developmental stages: E12-E18 and P0, P4, P8, P14, P21 and P29 postnatal mouse brain. Curves were fitted (line plots) to bar values using a log normal peak equation. The analysis shows an earlier peak of Neto2 mRNA during development compared with Neto. After P14 both present similar levels of expression.

Figure 3. Amplitude and Desensitization



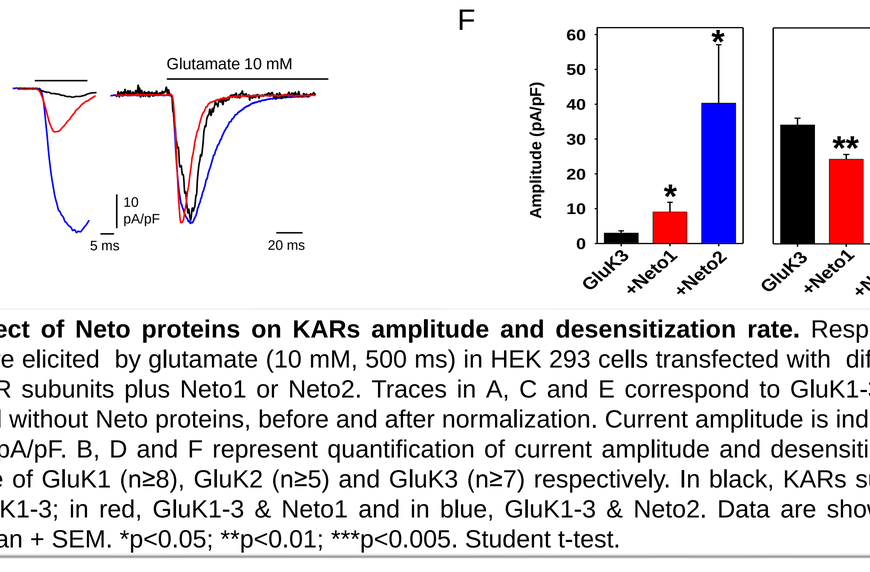
Effect of Neto proteins on KARs amplitude and desensitization rate. Responses were elicited by glutamate (10 mM, 500 ms) in HEK 293 cells transfected with different KAR subunits plus Neto1 or Neto2. Traces in A, C and E correspond to GluK1-3 with and without Neto proteins, before and after normalization. Current amplitude is indicated as pA/pF. B, D and F represent quantification of current amplitude and desensitization rate of GluK1 (n≥8), GluK2 (n≥5) and GluK3 (n≥7) respectively. In black, KARs subunit GluK1-3; in red, GluK1-3 & Neto1 and in blue, GluK1-3 & Neto2. Data are shown as mean + SEM. *p<0.05; **p<0.01; ***p<0.005. Student t-test.

Figure 4. Agonist Affinity



KARs affinity is modified by coexpresion of Neto proteins. HEK293 cells were transfected with GluK1-3 and Neto1 or Neto2. Dose response curves were calculated in each case by fitting data to the logistic equation. A, C and E show traces for GluK1-3 KAR subunits alone or in combination with Neto1 or Neto2 of different glutamate concentration. B, D and F show dose response curves for GluK1, GluK2 ad GluK3, respectively, where Neto1 and Neto2 increase affinity of KAR complexes. In the case of GluK3 EC50 was not calculated because 30 mM glutamate was not saturating. In black color GluK1-3 receptor without auxiliary proteins. Red and blue correspond to the action of NETO1 and NETO2, respectively.

Figure 5. Desensitization Recovery



Variable effect of Neto proteins in desensitization recovery rate. Recovery from desensitization was measured in HEK 293 cells transfected with KAR plus Neto proteins. KARs complexes were activated by a 500 ms pulse of 10 mM gutamate at different intervals. In A, C and E traces from GluK1-3 KAR (black) superimposed with responses obtained when coexpressing Neto1 or Neto2 (red and blue, respectively). Responses to glutamate were evoked at 500 ms intervals. In B, D and F, plots show the kinetics of recovery from desensitization. Data are mean + SEM. *p<0.05; **p<0.01Student t-test.

Figure 6. Sodium Dependence of Channel Gating

Neto proteins modify the dependence for Na^+ of channel gating in different KARs. KARs transfected HEK293 cells with (red for Neto1 and blue for Neto2) and without Neto proteins (in black) were tested in a Na^+-free Cs^+-containing external solution. Responses were elicited by 10 mM glutamate pulses. A and C show examples of traces of GluK1 and GluK2 respectively, in the homomeric or in heteromeric complexes with GluK5. B and D show the quantification of current remaining in the absence of Na^+. Homomeric (left plot) and heteromeric (right plot). Data are mean + SEM, n≥4. *p<0.05; **p<0.01; ***p<0.005 student t-test.

Data Summary

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.

	Current amplitude		Desensitization τ		Agonist affinity		Recovery Rate from desensitization		Sodium dependence (current loss)	
	Neto1	Neto2	Neto1	Neto2	Neto1	Neto2	Neto1	Neto2	Neto1	Neto2
GluK1	282%	573%	43%	242%	2898%	758%	625%	95%	58%	31%
GluK2	143%	146%	125%	213%	433%	289%	370%	120%	42%	27%
GluK3	303%	1348%	71%	148%	↑	↑	270%	89%	nd	nd