IMMUNOPHARMACOLOGICAL CHARACTERIZATION OF PRESYNAPTIC RELEASE-REGUALTING AMPA AUTO RECEPTORS IN THE CORTEX OF MICE

Francesca Cisani[1] , Matteo Vergassola[1] , Guendalina Olivero[1] , Cesare Usai[2] , Anna Pittaluga[1]

[1] Department of pharmacy, DIFAR, University degli Studi di Genova, Genoa - Italy, [2] National Research Council, Institute of Biophysics, Genoa - Italy

Introduction: In recent year we demonstrated that antibodies recognizing the outer sequence of receptor subunit proteins permit the pharmacological characterization of the presyanptic release-regulating receptors in isolated nerve endings (Olivero et al., 2019). This study was recently extended to AMPA receptors and aims at characterizing the subunit composition of these receptors in the cortex of adult mice.

Methods: Cortical synaptosomes were incubated with one of the following antibodies: rabbit anti-GluA1 (1:500) or rabbit anti-GluA2 (1:500) or mouse anti-GluA3 (1:500) or rabbit anti-GluA4 (1:500) and then labelled with a radioactive tracer, i.e. [3H] D-aspartate, which allows to monitor the exocytotic-like release of glutamate from nerve endings. Synaptosomes were up-down superfused and tritium exocytosis elicited by exposing them to a mild depolarizing stimulus (50 uM AMPA /1 uM cyclothiazide, for 90 seconds). Four superfusate fractions were collected and measured for radioactivity to quantify the AMPA-evoked release of glutamate. Confocal microsopy and western blot analysis was performed to support by a biochemical point of view the functional observations.

Results:

Bichemical results

Confocal microscopy demonstrated a diffuse colocalization of AMPA receptor subunits (namely GluA1, GluA2, GluA3 and GluA4 receptor proteins) with syntaxin-1A, consistent with the presynaptic expression of the AMPA receptors. To verify the presence of the AMPA subunits on glutamatergic nerve endings, we also performed confocal microscopy using VGLUT-1 (vesicular glutamate transporter-1) as a marker for glutamatergic terminals. Again, a diffuse GluA1, GluA2, GluA3 and GluA4-immunoreactivity was observed in VGLUT-1 immunopositive cortical synaptosomes. In a whole these results indicate the presence of AMPA receptor subunits in presyanptic glutamatergic particles isolated from the cortex of adult mice.

Functional results

Synaptosomes were incubated in the absence or in the presence of anti–GluA1, anti-GluA2, anti-GluA3 and anti-GluA4 antibodies (one antibody for each preparation) and the release of preloaded [3H]-D-Asp elicited by AMPA (50μM) in the presence of ciclotiazide (10μM) quantified. The results showed a significant increase of the AMPA-evoked [3H]-D-Asp in cortical synaptosomes incubated with anti-GluA3 when compared to antibody untreated control. Differently, the AMPA-evoked [3H]-D-Asp release from synaptosome incubated with anti-GluA1, and anti-GluA4 antibodies was unchanged when compared to control. Further studies are required to define the impact of anti-GluA2 antibodies on the same functional paradigm.

Discussion and conclusion: We have demonstrated that AMPA receptors exist in cortical glutamatergic synaptosomes and control glutamate release. A significant increase in glutamate release was observed when cortical synaptosomes were incubated with anti-GluA3. The mechanism at the basis of this functional modification remains so far unexplored. Rasmussen's encephalitis is a rare chronic neurological disorder characterized by unilateral inflammation of the cerebral cortex, drug-resistant epilepsy, and progressive neurological and cognitive deterioration. The disease is typified by high levels of antibody directed against the GluA3 subunit of AMPA receptors. Given that one of the hallmarks of Rasmussen's encephalitis is abnormally high synaptic glutamate release, resulting in excitotoxicity, we propose that the auto-antibody anti-GluA3, by increasing the release of AMPA-evoked glutamate release, can have a role in this detrimental event.