Ca^{2+} permeable AMPA receptors evoke glutamate release from Bergmann glia processes

D. Frattaroli[1], G. Maura[1,3], M. Passalacqua[2,3], S. Alloisio[4], M. Nobile[4], C. Cervetto[1,3], M. Marcoli[1,3]

[1]Dept. of Pharmacy, Pharmacology and Toxicology Section, University of Genoa, Italy; [2]Dept. of Experimental Medicine, Biochemistry Section, University of Genoa, Italy; [3]Center of Excellence for Biomedical Research CEBR, University of Genoa, Italy; [4] Institute of Biophysics, CNR, Genoa, Italy

Bergmann cells represent the major glial cell type in the cerebellar cortex and are increasingly recognized to play active roles in the control of glutamatergic transmission onto Purkinje cells. Expression of Ca^{2+}-permeable AMPA receptors, lacking the GluA2 subunit, is a characteristic feature of Bergmann glia: the receptors are sited on Bergmann cell bodies and along processes that encapsulate the synapses between parallel or climbing fibers and Purkinje cells, showing preferential distribution in the processes surrounding synapses and in particular on the plasma membrane facing the presynaptic ending of parallel fibers. Activation of the Ca^{2+}-permeable AMPA receptors on Bergmann processes and the consequent Ca^{2+} signaling is recognized as being essential to maintain structural and functional connections between the glial cell and the glutamatergic synapses. Nevertheless, the impact of Bergmann glia Ca^{2+} signaling on synaptic function, and the mechanisms mediating modulation of synaptic glutamatergic transmission, are not fully understood. In particular, while it is generally recognized that Ca^{2+} signaling in astrocyte processes may trigger release of gliotransmitters, to our knowledge there is no information if local Ca^{2+} responses triggers release of glutamate or of other gliotransmitters from Bergmann glia.

To better understand the mechanisms that might be involved in the modulation of synaptic transmission by Bergmann processes, herein we investigate Ca^{2+} responses and gliotransmitter release from purified astrocytic processes (gliosomes) acutely prepared from adult rat cerebellum.

Immunocytochemical confocal and Western blot analysis confirmed that cerebellar GFAP-positive gliosomes are a purified preparation of glial processes; more then 80% of gliosomes (positive for Brain-specific Lipid Binding Protein, BLBP) derived from Bergmann cells. In experiments on superfused gliosomes we obtained evidence for the presence of glutamate release-facilitatory AMPA receptors (AMPARs). The AMPA-evoked [^{3}H]D-aspartate or glutamate release depended on Ca^{2+} entry, as it was abolished in Ca^{2+}-free medium. Direct evidence that Ca^{2+} enters the processes following AMPA receptor activation was obtained by measuring Ca^{2+} signals in processes. Ineffectiveness of the wide-spectrum blocker of voltage-dependent Ca^{2+} channels $CdCl_2$ on both Ca^{2+} signals and glutamate release indicated that AMPA-evoked Ca^{2+} entry did not involve opening of the voltage-dependent Ca^{2+} channels. Sensitivity of the AMPA-evoked Ca^{2+} signal or glutamate release to NASPM, a selective inhibitor of GluA2-lacking Ca^{2+}-permeable AMPA receptors) is consistent with Ca^{2+} entering the astrocyte processes through the AMPA receptors. The AMPA-evoked glutamate release appeared due to activation of vesicular exocytotic release, as inhibition of vesicular loading by the vesicular glutamate transporter (VGLUT) inhibitors Rose Bengal or Trypan Blue or by the H^+-ATPase inhibitor bafilomycin A1 prevented the AMPA-evoked glutamate release. Confocal analysis confirmed that BLBP- and GFAP-positive processes expressed VGLUT1 and VGLUT2.

We conclude that: - viable Bergmann glia processes can be prepared from adult rat cerebellum; - a vesicular mechanism for release of glutamate is present in mature Bergmann processes; - entry of Ca^{2+} trough AMPA receptors located on Bergmann processes is coupled with vesicular glutamate release. Finally, we propose that the activation of vesicular glutamate release by Ca^{2+}-permeable AMPA receptors might be a mechanism contributing to the regulation of glutamatergic synapses by Bergmann glia processes.

Supported by University of Genoa (to C.C.)