Activation of astroglial calcineurin during neuronal activity and LTP

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Astrocytes communicate with neurons and respond to neuronal activity by increases of intracellular Ca^{2+}, release of gliotransmitters and by morphological changes, directed to adaptation of astrocytes to the increased synaptic activity. Although Ca^{2+} elevations, mediated by astroglial mGluR5, have been implicated in astrocyte-neuronal crosstalk and in structural plasticity of astrocytes, molecular mechanisms of these changes are currently unknown. We hypothesized that a Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CaN), which is responsible for remodeling of astroglial Ca^{2+} signaling toolkit in neurodegenerative diseases such as Alzheimer's disease, may be a mediator of astroglial plasticity. We tested this hypothesis employing lentiviral delivery of chimeric construct NFATΔ-GFP, which is used as a sensor of CaN activation. We transduced with NFATΔ-GFP murine hippocampal mixed astrocyte-neuronal primary cultures and monitored nuclear translocation of the probe in astrocytes after induction of neuronal activity or long-term potentiation (LTP). Treatment of mixed astrocyte-neuronal, but not of pure astroglial cultures, with bicuculline (20 μM), an antagonist of inhibitory $GABA_A$ receptors, known to induce an *in vitro* status epilepticus, induced nuclear translocation of NFATΔ-GFP in astrocytes after 45-60 min of treatment. Furthermore, application of a protocol, known to induce chemical LTP (cLTP) (application of 1 μM strychnine, 200 μM glicine and 20 μM bicuculline in Mg2+-free saline for 4 min, following by incubating in Ca2+ and Mg2+-containing saline), induced robust translocation of NFATΔ-GFP in the nucleus of astrocytes 10-60 min after the washout. The same protocol was ineffective in pure astroglial cultures. In both stimulation protocols (bicuculline and cLTP), the NFATΔ-GFP translocation was prevented by CaN inhibitors FK506 (100 nM) and cyclosporine A (500 nM). Analysis of Ca2+ signals using Fura-2 revealed elevation of Ca2+ in astrocytes but not in neurons 5-30 min after application of cLTP paradigm. Both, Ca^{2+} signals and NFATΔ-GFP translocation in astrocytes were blocked i) when cultures were challenged with cLTP paradigm in presence of MK801 (inhibitor of NMDA receptors), ii) when Ca^{2+}-free medium or iii) inhibitors of store-operated calcium entry (SOCE, 2APB and Pyr3) were applied after cLTP induction. We conclude that CaN is activated in astrocytes in response to neuronal activity through Ca^{2+} entry via store-operated mechanism, and speculate that it may mediate adaptational processes of astroglial plasticity.