Translational Research and Drug Development

Intersectin 1 interacts with STOP protein in neurons

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Aim: Endocytosis is a complex multistep process that requires organization of multiprotein complexes with strictly determined composition. Formation of such complexes is coordinated by endocytic adaptor proteins. One of these proteins, intersectin 1, has been implicated in development of Down syndrome and Alzheimer's disease. Our previous studies have shown that in neurons specific intersectin 1 isoforms are expressed comparing to other cell types. The aim of this study was to find novel intersectin1 binding partners in neurons.
Methods: Molecular cloning, recombinant protein expression and purification, pull-down assays, MALDI-TOF mass spectrometry, immunoprecipitation, transfection of rat hippocampal neurons primary culture, fluorescent laser scanning confocal imaging.
Results: Recombinant GST-fused SH3 domains of intersectin 1 were expressed in bacteria and used as bait in in vitro binding assay with mouse brain total protein lysate. For SH3A domain we detected an unknown binding protein of 125 kDa. This protein was identified by MALDI-TOF mass spectrometry as STOP (stable tubule-only polypeptide). Since glial cells contain STOP isoforms with smaller molecular weight, we concluded that STOP is neuron-specific binding partner of intersectin 1.Results on mass spectrometry were confirmed by GST-pull-down of SH3 domains with mouse brain total protein lysate and subsequent Western blot hybridization of bound proteins with monoclonal anti-STOP antibodies. Formation of STOP-intersectin 1 complex *in vivo* was proved by co-immunoprecipitation using anti-STOP and anti-intersectin 1 antibodies. Finally, we found that these two proteins co-localize in rat primary hippocampal neurons both in soma and neurites.
Conclusions: We have identified novel intersectin 1-binding protein STOP and confirmed our results by several independent methods.

Amniotic fluid soluble Toll-like receptor 4 in pregnancies complicated by preterm prelabor rupture of the membranes

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Aim: To determine amniotic fluid soluble Toll-like receptor 4 (sTLR4) levels in women with preterm prelabor rupture of the membranes according to the presence of microbial invasion of the amniotic cavity and histological chorioamnionitis and its relation to neonatal outcome.

Methods: One hundred two women with singleton pregnancies with a gestational age between 24+0 and 36+6 weeks were included in a prospective cohort study. Amniocenteses were performed, and the concentrations of sTLR4 in the amniotic fluid were determined using sandwich enzyme-linked immunosorbent assay technique.

Results: Women with the presence of microbial invasion of the amniotic cavity had higher sTLR4 levels [median 54.2 ng/mL, interquartile range (IQR) 10.15-289.9] than those without this condition (median 18.1 ng/mL, IQR 8.1-29.9; p = 0.001). Women with the presence of histological chorioamnionitis had a higher sTLR4 level (median 28.0 ng/mL, IQR 11.15-178.1) compared with women without histological chorioamnionitis (median 13.0 ng/mL, IQR 7.8-28.7; p = 0.003). A mixed linear model was used to adjust for confounders. The difference was found only between women with and without microbial invasion of the amniotic cavity in this model.

Conclusions: Microbial invasion of the amniotic cavity was associated with higher amniotic fluid sTLR4 levels independent of confounders.

Key words: Histological chorioamnionitis, microbial invasion of the amniotic cavity, preterm prelabor rupture of the membranes, sTLR4

Neuroprotection by reduction of membrane cholesterol content

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Aim: In stroke, cerebral hypoxia/ischemia, traumatic brain injury, neurotoxic consequences are evoked by enhanced extracellular concentration of glutamate, which is the major excitatory neurotransmitter in the CNS, and transporter-mediated release of glutamate (glutamate uptake reversal) is the main mechanism of glutamate release. Under these pathological conditions, cholesterol-lowering drugs statins have neuroprotective features. The aim of this study was to assess transporter-mediated release of glutamate from rat brain nerve terminals (synaptosomes) under conditions of cholesterol deficiency.
Methods: Laser scanning confocal microscopy with fluorescent dye filipin, spectrofluorimetry with pH-sensitive fluorescent dye, radiolabeled assay (L-[14C]glutamate), glutamate dehydrogenase assay.
Results: Cholesterol acceptor methyl-β--cyclodextrin (MβCD) reduced the cholesterol concentration in synaptosomes by 25%. Under conditions of cholesterol deficiency: 1) tonic release of endogenous glutamate; 2) stimulated by depolarization of the plasma membrane transporter-dependent glutamate release; 3) release of glutamate by means of heteroexchange with competitive transported inhibitor of glutamate transporters DL-threo-β--hydroxyaspartate; 4) transporter-mediated glutamate release evoked by protonophore FCCP; 5) glutamate release in low-Na medium; was decreased, whereas the endogenous extracellular level of glutamate was increased.
Conclusions: Decrease in the level of membrane cholesterol may be used for neuroprotection under pathological conditions including stroke, cerebral hypoxia/ischemia, traumatic brain injury that were associated with an increase in glutamate uptake reversal. Visa versa in norm, a decrease in the concentration of membrane cholesterol may cause neurotoxic consequences resulted from the enhancement of the extracellular glutamate level because of a decrease in glutamate uptake.
Keywords: synaptosomes, methyl-β--cyclodextrin, cholesterol deficiency, glutamate release