Ammonium-induced GS degradation undergo proteasome independent pathways

Sari Kishikawa, Miho Terunuma

Division of Oral Biochemistry, Graduate School of Medical and Dental Sciences, Niigata University, Japan

Ammonia produced from various tissues is released into the blood and mainly metabolized by the liver. However, a small amount of ammonia is metabolized in the brain by the cells called astrocytes. Astrocytes, one of the major cells in the central nervous system, are the only cells in the brain that remove ammonium using enzyme called glutamine synthetase (GS). Furthermore, glutamate, which triggers excessive nerve excitation and causes neuronal cell death is removed from the extracellular space by astrocytes to prevent the neurotoxicity. Interestingly, ammonium helps to convert glutamate to glutamine.

For many years, it has been reported that the expression of GS is decreased under high ammonium state but the degradation system of GS is still unclear.

We have previously identified that inhibitory neurotransmitter receptor called GABAB receptors associate with GS and protect GS from proteasomal degradation (Huyghe et al., J Biol Chem, 2014). These results suggested that inhibitory neurotransmitter GABA not only reduces neuronal activity but also regulates glutamate metabolisms in astrocytes. Since ammonia requires glutamate to convert it to glutamine, we examined if GS-GABABR complex plays role in ammonia metabolism using rat cultured cortical astrocytes.

Prolonged 10mM ammonium chloride (NH₄Cl) stimulation, a pathological concentrations of NH₄Cl which mimics concentration of hyperammonemia, significantly reduced GS levels, and it was accelerated by L-glutamate application. The down-regulation of GS was not recovered neither by protease inhibitor leupeptin nor ubiquitin-proteasome inhibitor MG132, therefore it was considered that ammonia-induced GS degradation undergo proteasome /lysosome independent pathways. We also observed that ammonia-induced GS degradation occur independently from GABAB receptor activation.

We are currently analyzing the cellular localization of GS and GABAB receptors, as well as the morphological changes of astrocytes.