Molecular mechanisms for ammonia-induced increase in chloride concentration in cultured hippocampal neurons

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Hyperammonemia is one of the most important factors in the pathogenesis of hepatic encephalopathy. In this study, we found that long term (24-48 hrs) exposure of MQAE-loaded hippocampal neurons to 2mM ammonia induced an elevation of the intracellular Cl^- level ($[Cl^-]i$) which was inhibited by anion (Cl^-/HCO_3^-) exchange inhibitors, SITS and DIDS. Ammonia also increased this exchanger's mRNA and protein level. Since these effects of ammonia were inhibited by the protein kinase C (PKC) inhibitors H-7 and calphostin C, we herein examined whether ammonia induces PKC activation or not, by Western blot analysis usig PKC subtype-specific antibodies, in cultured rat fetal hippocampal neurons. Treatment with 2mM NH_4Cl for 5-30min time-dependently increased the immunoreactivities of both α-and βII- PKCs in the particulate fractions with decreases in the cytosol fractions, indicating the translocations of α-and βII- PKCs from cytosol to the membranes. Furthermore, stimulation by 2mM NH_4Cl resulted in a 1.5- to 2-fold elevation of intracellular Ca^{2+} levels ($[Ca^{2+}]i$) in fura-2 AM-loaded neuronal cells in a time course pararell with PKC activations.

From these results, ammonia treatment appears to activate Ca^{2+}dependent α-and βII-PKCs in hippocampal neurons, which probably induces $[Cl^-]i$ elevation through enhanced expression of the anion exchanger.