Cl^- pump and its pathophysiological changes in the brain

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Summary

As a candidate for outwardly directed active Cl^- transporters, we previously reported ATP-dependent and phosphatidylinositol-4-monophosphate (PI4P)-stimulated Cl^- pump/ATPase (J Biol Chem 264,17416,1989**,** Brain Res 641, 164 ,1994), and isolated 520 kDa protein complex with the Cl^- pump activity (Neurosci Lett 258, 85 (1998)). The cDNA of 55 kDa subunit was cloned (Biochem Biophys Res Commun 289,363, 2001). In this study, we examined the structure of Cl^- pump/ATPase as well as its involvement in pathophysiology of Alzheimer's disease.

(1) Cl^- pump/ATPase 51 kDa subunit

SDS-PAGE of 520 kDa Cl^- pump/ATPase protein complex yielded 51, 55, 60 and 62 kDa proteins. A catalytic subunit 51 kDa protein was isolated using reactive red column, SDS-PAGE and 2nd dimension electrophoresis. In Edman degradation, the N-terminal amino acid appeared to be blocked. Mass spectrometry (TOF/MS) yielded no matching with any known protein data base. Further analysis of the 51 kDa protein is now underway with a possibility that this protein is one not yet reported previously.

(2) Molecular mechanisms of neuronal dell death related to amyloid β (Aβ)-induced inhibition of Cl^- pump/ATPase activity

i) Aβ(25-35)-induced enhancement of glutamate neurotoxicity is dependent on Cl^-.

Replacement of Cl^- in culture medium with isethionate abolished Aβ-induced increase in neuronal $[Cl^-]i$ resulted from inhibition of Cl^- pump/ATPase activity. Under such conditions, Aβ-induced enhancement of glutamate toxicity was also blocked as assayed by mitochondrial WST-reducing activity and LDH relaese, suggesting that the enhancement of the toxicity is Cl^--dependent.

ii) Molecular mechanisms of Cl^--dependent enhancement of glutamate toxicity:

Aβ-induced enhancement of glutamate toxicity was associated with increased tyrosine phosphorylation of 110 kDa, 76 kDa and 60 kDa proteins. Tyrosine phosphorylation of these proteins was attenuated in the culture using low Cl^- medium. Protein analyses using TOF/MS spetrometry and specific antibodies suggest that the Cl^--dependently phosphrylated proteins are Src-related ones.