A chloride ion pump (Cl^- pump) in brain and kidney

Chiyoko Inagaki, Naoki Hattori, Toshiaki Higashida and Xun-Ting Zeng
Department of Pharmacology, Kansai Medical University

The chloride ion concentration in neurons of the central nervous system such as spinal motoneurons and hippocampal pyramidal cells is lower than that predicted from passive distribution. This enables inhibitory hyperpolarizing responses to occur when transmitter operated chloride channels are facilitated. As a canditate for an active chloride transporting system that produces such an inwardly directed chloride gradients, we found an ATP-dependent chlorideextrusion system, Cl^- pump, in the brain (J.Biol.Chem.1989). Later, the activity was also found in the kidney. Recently, we isolated 520 kDa protein with Cl^- pump activity. In this study, we analyzed subunit structure and localization of Cl^- pump using anti-Cl^- pump antibody.

(1) Cl^- pump protein (520 kDa) was isolated from plasma membrane fractions of rat brain, and administered subcutaneously to rabbits for immunization. Anti-Cl^- pump antibody reacted with 51 kDa subunit of Cl^- pump and inhibited both Cl^--ATPase and ATP-dependent $^{36}Cl^-$ transport (Cl^- pump) activities. (2) Cl^- pump-like immunoreactivity was observed in the plasma membranes of brain neurons such as cerebral cortical pyramidal cells and cerebellar Purkinje cells, and those of basal plasma membranes of intercalated cells of renal collecting ducts. (3) Electrophoresis (SDS-PAGE) of Cl^- pump protein yielded at least 3 peptide bands including immunoreactive one (51 kDa) and that with an N-terminal amino acid sequence that has not been reported previously.

It is suggested that Cl^- pump has multisubunit structure with a possible ative subunit of 51 kDa peptide, and is localized in plasma membranes of brain neurons and intercalated cells of renal collecting ducts.