RECONSTITUTION AND PARTIAL PURIFICATION OF Na^+-DEPENDENT GLUTAMATE TRANSPORTER FROM CANINE BRAIN AND ANALYSIS OF MOLECULAR POLYMORPHISM

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The Na^+-dependent L-glutamate transporter (Na/Glu transporter) of synaptic membranes in canine cerebrum was solubilized with 3-[(3-chloramidepropyl)dimethyl ammonio]-1-propanesulfonic acid (CHAPS) and incorporated into proteoliposomes consisting of soybean lecithin and canine brain lipids by a reconstitution procedure involving rapid gel filtration. Utilizing this procedure, the effluent of chromatographic separation of the solubilized synaptic membrane proteins was assayed for their L-[^{3}H]glutamate uptake into proteoliposomes. A 32.4-fold increase in the specific activity of the Na/Glu transporter and about an 4,000-fold increase at the protein level were achieved by a series of chromatographies on hydroxylapatite, Q Sepharose, wheat germ agglutinin Sepharose, and Superose 6 columns. A rapid inactivation of the solubilized transporter is likely to be the cause of a rather low increase in the specific activity. Partially purified Na/Glu transporter mainly contained polypeptides with apparent molecular masses of 110,000 and 55,000 and many other minor proteins.

The Na/Glu transport activity was divided into two or more peaks in each step of the purification described above, indicating the presence of some different transporter molecules. To assess the molecular polymorphism of the Na/Glu transporter, cross-inhibition of L-[^{3}H]Glu transport with several glutamate derivatives was performed in cerebrum, cerebellum, and erythrocytes. D-Aspartate and dihydrokainate inhibited L-[^{3}H]Glu transport in the order cerebrum$>$cerebellum$>>$erythrocytes, while other derivatives, including D-glutamate, L-aspartate, and threo-3-hydroxy aspartate, showed almost the same inhibitory effect on the transport in these different organs. Moreover, this study also demonstrated that the Na/Glu transporter in both brain and erythrocytes definitely required intracellular K^+ in addition to an inward gradient of Na^+. These results indicate that there are various isoforms of the Na/Glu transporter in different tissues and cells with the same stoichiometry in the transport of Na^+, K^+, and glutamate.