Current Topics

Taste and Health: Nutritional and Physiological Significance of Taste Substances in Daily Foods

A Vesicular Transporter That Mediates Aspartate and Glutamate Neurotransmission

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Aspartate, an excitatory amino acid, is known to be stored in synaptic vesicles and exocytosed from some neurons to perform aspartergic neurotransmission. Through *in vitro* reconstitution, we found that sialin, a lysosomal sialic acid exporter, is responsible for the vesicular storage of aspartate in hippocampal neurons and pinealocytes. Mutations found in Salla disease cause decreased aspartate transport activity without affecting sialic acid transport. Thus, sialin is a multifunctional transporter. It is possible that people with Salla disease lose the ability of aspartergic neurotransmission, and this could explain why Salla disease involves severe neurological defects.

Key words aspartate; neurotransmission; Salla disease; vesicular excitatory amino acid transporter; vesicular glutamate transporter

1. INTRODUCTION

Glutamate and aspartate are major components in KONBU DASHI and act as excitatory neurotransmitters. Glutamate is stored in synaptic vesicles after active transport by vesicular glutamate transporters (VGLUTs), and exocytosed, and binds to a variety of glutamate receptors, leading to glutamatergic neurotransmission.^{1,2)} Like glutamate, aspartate is usually co-stored with glutamate in hippocampal synaptic vesicles and pineal synaptic-like microvesicles (SLMV), and exocytosed from neurons upon stimulation, and stimulates neighboring cells upon binding to *N*-methyl D-aspartate (NMDA) receptors^{3,4)} (Fig. 1). Although these findings supported the occurrence of aspartergic neurotransmission, this process remained controversial because the transporter responsible for the vesicular storage of aspartate was unknown. VGLUTs, one of the candidates for vesicular aspartate transporter, do not recognize aspartate as a transport substrate.

2. ESTABLISHMENT OF AN *IN VITRO* RECONSTITUTION METHOD

Recently, we established a general procedure for analyzing the structures and functions of transporters, which comprises the expression of transporters in insect cells, purification and reconstitution into liposomes.⁵⁾ Using this procedure, we can quantify the transport activities of all types of transporters including mutants and single nucleotide polymorphisms (SNPs) from any species. For example, we have identified the essential amino acid residues of VGLUTs, and revealed the binding site for glutamate by constructing a 3D structural model. Because this essential amino acid residue is conserved in the solute carrier (SLC17) family, small spatial changes near the binding region may determine the substrate

specificities of SLC17 family members. Thus, it seems that a subgroup other than the VGLUTs is involved in the vesicular storage of aspartate. Sialin (SLC17A5), a lysosomal H⁺/sialic acid cotransporter, is the most appropriate candidate because this protein is present in hippocampal synaptic vesicles and pineal SLMVs, as seen on immunoelectron microscopy (Fig. 2). Furthermore, selective loss of sialin expression due to RNA interference (RNAi) against sialin in pinealocytes decreases aspartate and glutamate exocytosis.

Fig. 1. Aspartergic Neurotransmission

Fig. 2. Vesicular Localization of Sialin in SLMVs

Double-labeling immunoelectron microscopy indicated that sialin is present in pineal SLMVs, after treatment with a pair of anti-synaptophysin monoclonal antibodies (5 nm particles, arrow), and anti-sialin polyclonal antibodies (10 nm particles, arrowheads) (Scale bar, 100 nm).

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Fig. 3. Sialin Acts as a Vesicular Aspartate/Glutamate Transporter

(A) Purified mouse sialin (10 μ g of protein) was visualized by Coomassie brilliant blue staining. (B) H^+ /Sialic acid cotransport by proteoliposomes (acidic outside). (C) $\Delta\psi$ -Dependent aspartate uptake. (D) $\Delta\psi$ -Dependent glutamate uptake.

We observed that RNAi treatment decreased the exocytosis of aspartate and glutamate to 32% and 60% of the control levels, respectively. These results suggest that sialin is responsible for the vesicular storage of aspartate and glutamate.

3. IDENTIFICATION OF A VESICULAR ASPARTATE TRANSPORTER

We expressed sialin in insect cells, and then solubilized, purified, and reconstituted it into liposomes. The purified sialin exhibited a single major polypeptide with an apparent molecular mass of 60 kDa (Fig. 3A). Upon reconstitution into liposomes, proteoliposomes showed Δ pH-dependent sialic acid transport activity, providing the purification of the active sialin (Fig. 3B). Upon formation of $\Delta\psi$ (positive inside), the proteoliposomes took up aspartate (Fig. 3C). A spectrum of possible *cis*-inhibitors suggested that aspartate uptake is insensitive to substrates of sialic acid transport but sensitive to aspartate and glutamate, as opposed to those of H^+ /sialic acid cotransport. Aspartate transport obligatorily required chloride anions and was inhibited by Evans blue, whereas H^+ /sialic acid cotransport did not require chloride anions and was insensitive to Evans blue. The proteoliposomes also took up glutamate in a similar manner in the case of aspartate transport (Fig. 3D). From these results, it was concluded that sialin acted as a vesicular aspartate and glutamate transporter with properties similar to but distinct from those of VGLUTs. It is noteworthy that other members of the SLC17 transporter family did not exhibit any aspartate/glutamate transport activity.

4. THE EFFECTS OF MUTANTS CAUSING SIALIC ACID STORAGE DISEASES

The biological importance of sialin has previously been demonstrated by the existence of sialic acid storage diseases. Mutations on the sialin gene are known to cause two diseases, infantile sialic acid storage disease (ISSD), an early fatal disorder with many features characteristic of a lysosomal disorder, and Salla disease, a neurological disorder in which the affected persons have a near-normal life expectancy^{6,7} (Figs. 4A,B). However, why different sialin mutations cause different diseases is less understood. We found that the sialin mutation causing Salla disease was completely

Fig. 4. Effects of the Mutation Causing Sialic Acid Storage Disease

(A) Topological model of sialin showing pathogenic mutations. (B) The symptoms of Salla disease and ISSD. (C) (Inset) Coomassie brilliant blue staining of the mutant protein (10 μ g). $\Delta\psi$ -Dependent aspartate/glutamate transport and H^+ /sialic acid cotransport activities. Statistical significance was determined by Student's *t*-test. * p <0.05; ** p <0.001.

inactive as to $\Delta\psi$ -dependent aspartate and glutamate uptake while sialic acid cotransport activity was retained. In contrast, the sialin mutation causing ISSD was active as to $\Delta\psi$ -dependent aspartate and glutamate uptake, whereas H^+ /sialic acid cotransport was completely absent (Fig. 4C). Thus, we propose that persons possessing sialin with the mutation that causes Salla disease exhibit impaired aspartergic neurotransmission rather than accumulation of sialic acid in lysosomes.

5. CONCLUSION

Through *in vitro* reconstitution, we identified a long searched for vesicular aspartate transporter.⁸ The transporter also transports glutamate, indicating that this protein is the fourth member of the vesicular glutamate transporter family. Because both glutamate and aspartate are excitatory amino acids, sialin should be named vesicular excitatory amino acid transporter (VEAT). We also found an unexpected link between aspartergic/glutamatergic neurotransmission and Salla disease. This finding may facilitate the identification of potential molecular targets for pharmacotherapy for this neurological disorder. Conversely, studies on the pathogenesis of Salla disease could provide clues for exploring the physiology of aspartergic/glutamatergic neurotransmission.

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