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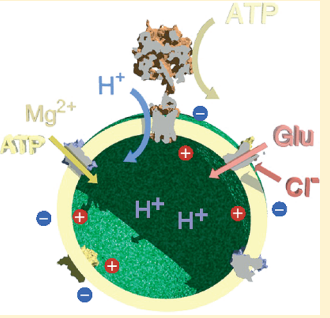
Vesicular Neurotransmitter Transporter: Bioenergetics and Regulation of Glutamate Transport

Hiroshi Omote,*[,†] Takaaki Miyaji,[‡] Narinobu Juge,[†] and Yoshinori Moriyama[†,‡]

[†]Department of Membrane Biochemistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8530, Japan

[‡]Advanced Science Research Center, Okayama University, Okayama 700-8530, Japan

ABSTRACT: Glutamate plays essential roles in chemical transmission as a major excitatory neurotransmitter. The accumulation of glutamate in secretory vesicles is mediated by vesicular glutamate transporters (VGLUTs) that together with the driving electrochemical gradient of protein influence the subsequent quantum release of glutamate and the function of higher-order neurons. The vesicular content of glutamate is well correlated with membrane potential ($\Delta\psi$), which suggests that $\Delta\psi$ determines the vesicular glutamate concentration. The transport of glutamate into secretory vesicles is highly dependent on Cl^-. This anion stimulates glutamate transport but is inhibitory at higher concentrations. Accumulating evidence indicates that Cl^- regulates glutamate transport through control of VGLUT activity and the H^+ electrochemical gradient. Recently, a comprehensive study demonstrated that Cl^- regulation of VGLUT is competitively inhibited by metabolic intermediates such as ketone bodies. It also showed that ketone bodies are effective in controlling epilepsy. These results suggest a correlation between metabolic state and higher-order brain function. We propose a novel function for Cl^- as a fundamental regulator for signal transmission.



Chemical transmission involves accumulation of neurotransmitters into secretory vesicles and subsequently their exocytosis to the extracellular space[1] (Figure 1). Such chemical transmission has fundamental roles in signal transduction in the neuronal network and in numerous peripheral non-neuronal systems. Among a large number of neurotransmitters, glutamate is a major excitatory neurotransmitter. Accumulation of glutamate in secretory vesicles is mediated by vesicular glutamate transporters (VGLUTs). This process is driven by an electrochemical gradient of H^+ established by V-ATPase. The driving force and VGLUT activity affect vesicular glutamate content and subsequent glutamatergic signaling. In this review, we describe the energetics and regulation of VGLUTs and related transporters.

VESICULAR NEUROTRANSMITTER TRANSPORTERS

Just as there is a variety of transmitters used in chemical transmission, various types of vesicular neurotransmitter transporters are involved in the accumulation of transmitters in secretory vesicles. So far, nine vesicular neurotransmitter transporters have been identified and are classified into three families on the basis of substrate specificity and amino acid sequence similarity (Table 1). These three families are SLC17, SLC18, and SLC32 and are dedicated to the uptake of anionic, cationic, and electroneutral neurotransmitters, respectively.[2−4] In addition to substrate specificity, the transporter families also differ in driving force, regulation, and other kinetic properties.

SLC17. The SLC17 family is involved in vesicular transport of anionic neurotransmitters such as glutamate and aspartate (Figure 2A and Table 1). This family contains nine members, including three vesicular glutamate transporters (VGLUT1, VGLUT2, and VGLUT3) and the vesicular excitatory amino acid transporter (VEAT).[2,5] In addition to these vesicular neurotransmitter transporters, four sodium-dependent phosphate transporters (NPT1, NPT3, NPT4, and NPT homologue) are included in this family. These NPTs are involved in voltage-driven organic anion transport for the elimination of toxic xenobiotics in the kidney and are not related to signal transmission. Although their substrate and localization are different, the structural and biochemical properties of all SLC17 family transporters are similar to each other.

Recently, the ninth member of this family was identified and named the vesicular nucleotide transporter (VNUT).[6] Although ATP is known as a neurotransmitter for purinergic signaling,[7] the molecule responsible for the transport of ATP into vesicles has not yet been identified. Through gene mining of the human genomic sequence, the novel gene, SLC17A9, was discovered. Given the fact that the SLC17A9 protein is localized in ATP-storing vesicles and that the substrates of all SLC17 transporters are organic anions, this protein is the most likely to be the vesicular nucleotide transporter. Extensive biochemical analysis of the reconstituted, purified SLC17A9 protein revealed that this protein functions as a vesicular nucleotide transporter.[6]

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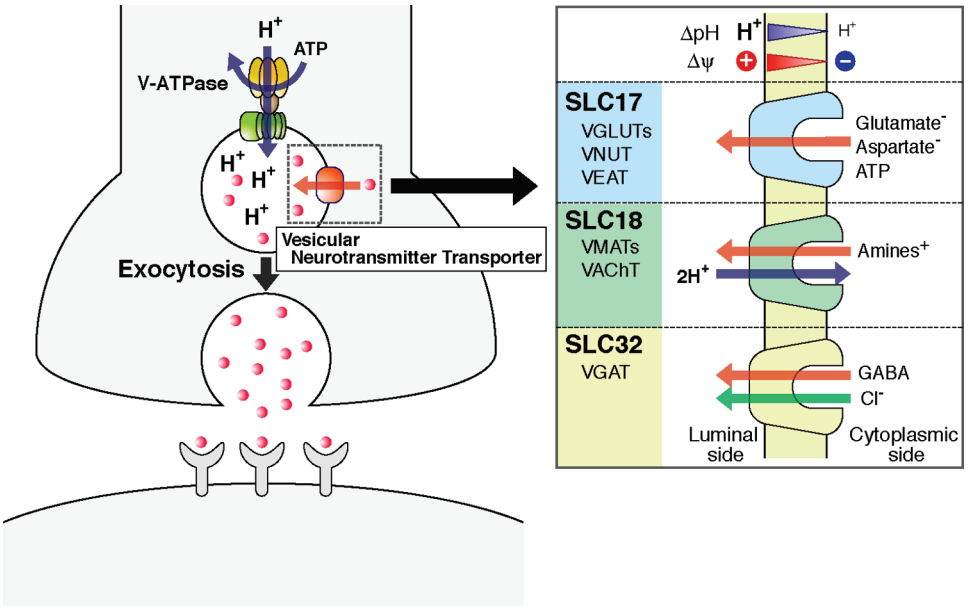

Figure 1. Chemical transmission and vesicular neurotransmitter transporters in synapses. The vesicular neurotransmitter transporter transports neurotransmitters into synaptic vesicles using an electrochemical gradient of H^+ established by V-ATPase. Upon stimulation, concentrated transmitters are released through exocytosis and bind to receptors on the postsynaptic membrane. The vesicular neurotransmitter transporters are categorized into SLC17, SLC18, and SLC32 families on the basis of their amino acid sequence similarities and substrate specificity.

Table 1. Vesicular Neurotransmitter Transporters

gene name	transporter	substrate	driving force and transport mode
SLC17A5	vesicular excitatory amino acid transporter (VEAT)	glutamate, aspartate	$\Delta\psi$, uniport
SLC17A6	vesicular glutamate transporter 2 (VGLUT2)	glutamate	$\Delta\psi$, uniport
SLC17A7	vesicular glutamate transporter 1 (VGLUT1)	glutamate	$\Delta\psi$, uniport
SLC17A8	vesicular glutamate transporter 3 (VGLUT3)	glutamate	$\Delta\psi$, uniport
SLC17A9	vesicular nucleotide transporter (VNUT)	ATP, ADP	$\Delta\psi$, uniport
SLC18A1	vesicular monoamine transporter 1 (VMAT1)	serotonin, adrenaline, noradorenaline, histamine, dopamine	$\Delta\psi$, ΔpH, H^+ antiport
SLC18A2	vesicular monoamine transporter 2 (VMAT2)	serotonin, adrenaline, noradorenaline, histamine, dopamine	$\Delta\psi$, ΔpH, H^+ antiport
SLC18A3	vesicular acetylcholine transporter (VAChT)	acetycholine	$\Delta\psi$, ΔpH, H^+ antiport
SLC32A1	vesicular GABA transporter (VGAT)	GABA, glycine	$\Delta\psi$, Cl^- cotransport

SLC18. SLC18 transporters are dedicated to the vesicular accumulation of cationic neurotransmitters such as acetylcholine, adrenaline, and serotonin[3,8] (Table 1). This family is composed of three members: vesicular monoamine transporter 1 (VMAT1), vesicular monoamine transporter 2 (VMAT2), and vesicular acetylcholine transporter (VAChT). VMAT1 is mainly expressed in peripheral endocrine cells such as adrenal gland. On the other hand, VMAT2 is mainly expressed in aminergic neurons in the central nervous system. The SLC18 transporters function as H^+ antiporters and have specific inhibitors, tetrabenazine for VMAT2, reserpine for VMAT1 and VMAT2, and vesamicol for VAChT. These inhibitors are good tools for kinetic analysis of SLC18 transporters.

SLC32. At present, the SLC32 family consists of only the vesicular GABA transporter (VGAT), which transports electrically neutral substrates such as GABA and glycine (Table 1). Because GABA and glycine work as inhibitory neurotransmitters, VGAT has been also given the name vesicular inhibitory amino acid transporter (VIAAT). Purified and reconstituted VGAT exhibits membrane potential-driven transport of GABA and glycine, suggesting the existence of a coupling ion.[9] GABA transport requires Cl^-, and GABA-dependent cotransport of Cl^- has been observed.[9] Because GABA transport is $\Delta\psi$-driven, cotransport of Cl^- most probably provides a significant part of the power stroke for VGAT. Recently, the structural similarity of VGAT to members of the SLC6 family was reported.[10] A SLC6 transporter is also reported to transport Cl^-, suggesting functional similarity to VGAT.[11]

PROPERTIES OF THE VESICULAR GLUTAMATE TRANSPORTER

Because glutamate functions as a major excitatory neurotransmitter, the mechanism of vesicular accumulation of glutamate is of major interest to neurobiochemists. Although biochemical analyses of isolated synaptic vesicles revealed ATP-dependent accumulation of glutamate, the molecule responsible for vesicular glutamate transport has not been identified.[12,13]

The first member of the SLC17 family to be isolated was the type 1 sodium-dependent phosphate transporter (NPT1) from kidney that takes up inorganic phosphate using a sodium gradient as the driving force.[14] Later a homologue, the brain-specific Na^+-

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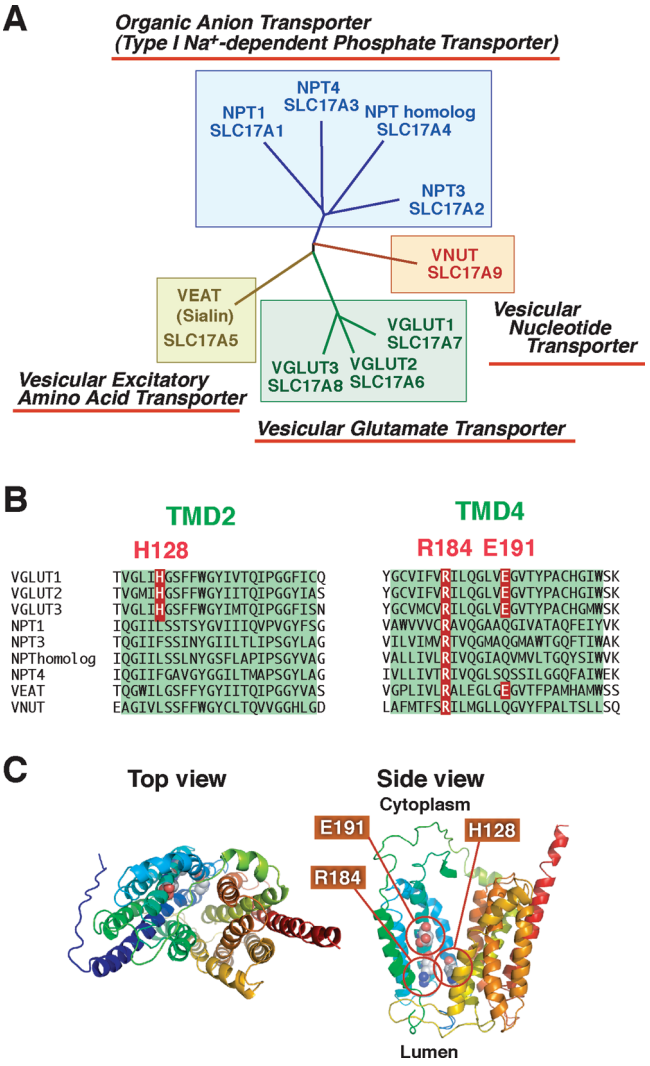

Figure 2. (A) Phylogenetic tree of the SLC17 transporter family. (B) Amino acid alignment of transmembrane domains 2 and 4. Amino acid residues essential for glutamate transport are colored red. (C) Top and side views of a structural model of VGLUT2.[31] The positions of three essential residues are indicated. For better viewing, transmembrane helices 1 and 5 were removed from the side view.

dependent phosphate transporter (BNPI), was identified.[15] Histochemical studies showed that BNPI is not localized in kidney, but rather to synaptic vesicles of glutamatergic neurons in the brain.[16] Biochemical studies in a heterologous expression system showed that BNPI exhibits glutamate transport activity with kinetic properties similar to those of synaptic vesicles.[17,18] Currently, BNPI is known as vesicular glutamate transporter 1 (VGLUT1). Shortly afterward, the differentiation-associated Na^+-dependent phosphate transporter (DNPI) was cloned and named vesicular glutamate transporter 2 (VGLUT2).[19−24] The third vesicular glutamate transporter was identified on the basis of sequence similarity.[25−27] VGLUT1 and VGLUT2 are expressed in glutamatergic neurons in a complementary manner, suggesting a functional differentiation of the two transporters.[20,22,24,28] In the central nervous system, VGLUT1 is mainly expressed in cerebral cortex, hippocampus, and cerebellar cortex, whereas VGLUT2 is mainly expressed in thalamus, brainstem, and deep cerebellar nuclei.[20,22,24,28] In contrast, VGLUT3 is expressed in cholinergic neurons in the dorsal and ventral striata and serotoninergic neurons in the raphe nuclei.[25−27,29] The nonglutamatergic distribution of VGLUT3 suggests a novel mode of glutamate signaling. Biochemical and kinetic properties of these isoforms are quite similar to each other, although VGLUT3 exhibit a slightly smaller K_m than VGLUT1 and VGLUT2.[27]

The functional importance of VGLUTs has been confirmed by knockout mouse studies.[30−35] VGLUT1 knockout mice are born in the expected Mendelian frequency but exhibit a progressive neurological phenotype, including blindness, uncoordination, and enhanced startle response.[30,31,33] As expected, isolated synaptic vesicles from these mice show a reduced rate of uptake of glutamate. These mice also exhibited a reduced level of glutamatergic neurotransmission in hippocampal neurons with decreased quantal size.[31] These studies clearly prove the functional importance of VGLUT1 in glutamatergic signaling in vivo. Genetic inactivation of VGLUT2 causes perinatal lethality consistent with predominant expression of VGLUT2 during embryonic and early postnatal development.[32,36] Recently, gene disruption of VGLUT3 revealed the involvement of VGLUT3 in auditory function and mechanical hypersensitivity.[34,35]

The kinetic properties of VGLUTs have been investigated using synaptic vesicles and vesicles isolated from heterologous expression systems. Inhibition of glutamate transport by ionophores demonstrated that the primary driving force of glutamate transport by VGLUT is membrane potential ($\Delta\psi$), although a significant contribution from the H^+ gradient (ΔpH) has been reported.[13,17,22−24,27,28,37−40] The apparent affinity to glutamate is around 1−5 mM, which is consistent with high cytoplasmic concentrations of glutamate.[13,17,22−24,26−28,37,39−43] Substrate specificity of VGLUT is strictly limited to glutamate; VGLUT recognizes neither aspartate nor glutamine.[13,38] Very strict substrate specificity is characteristic of VGLUTs and is not found in other SLC17 transporters. Another striking feature of VGLUT is its absolute requirement for Cl^- and biphasic Cl^- dependence.[13,17,23,24,37−40,43,44] At low Cl^- concentrations, glutamate transport is negligible and an increasing Cl^- concentration activates transport. Maximal activity is reached at ~4 mM (Figure 3). At higher Cl^- concentrations, transport activity gradually decreases. The chloride dependence of VGLUT is complex because of the absolute requirement for Cl^- by the VGLUT and the Cl^--dependent changes in $\Delta\psi$ and ΔpH. This issue is discussed in a later section.

The establishment of a new assay system using reconstituted, purified VGLUT clearly demonstrated the kinetic properties of the transporter.[39,40] The combination of overexpression and reconstitution of purified VGLUT also allows detailed mutagenic analysis.[39] Systematic mutation of conserved hydrophilic residues in the transmembrane regions of VGLUT2 showed that His128, Arg184, and Glu191 are essential for glutamate transport (ref 39 and Figure 2B,C). These residues are located in the putative substrate binding pocket of the structural model based on the crystal structure of bacterial glycerol-3-phosphate transporter (Figure 2C). Greatly reduced V_{max} values of these mutants and the structural model suggest the involvement of these residues in the translocation step of glutamate transport. Of these three essential residues, Arg184 is the most important because the mutational effect is more severe than that of His128 and Glu191. This residue is conserved in all SLC17 family members, suggesting a common essential role(s) such as the Cl^- activation process (Figure 2B).

Another interesting property of VGLUT is its multitransport activity. As described earlier, the first VGLUTs to be cloned were

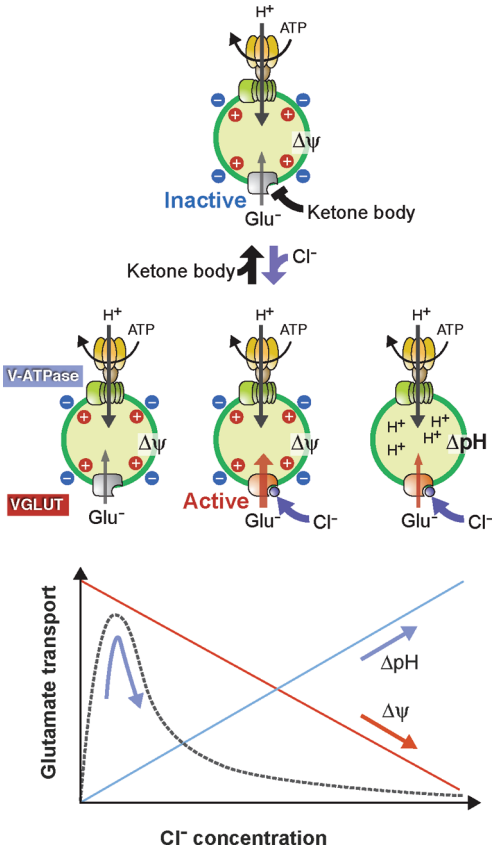

Figure 3. Cl^- dependence and regulation of glutamate transport. Cl^- regulates glutamate transport by controlling $\Delta\mu H^+$ and VGLUT activity. With an increasing Cl^- concentration, ΔpH is increased and $\Delta\psi$ is decreased. VGLUT exhibits a biphasic glutamate transport dependence on Cl^- concentration. Cl^- activates VGLUT activity at low concentrations and inhibits VGLUT at higher concentrations because of the loss of driving force. Ketone bodies inactivate VGLUT by inhibiting Cl^- binding.

characterized as inorganic phosphate transporters. Actually, purified VGLUT2 exhibits Na^+ gradient-driven inorganic phosphate transport activity. However, this activity is not inhibited by Evans blue, an inhibitor of glutamate transport, or excess glutamate. Glutamate transport activity requires Cl^-, but phosphate transport activity does not. Site-directed mutagenesis of three amino acid residues that are essential for glutamate transport does not affect phosphate transport activity. These results point out that the mechanisms of $\Delta\psi$-dependent glutamate transport and Na^+ gradient-driven inorganic phosphate transport are different. Thus, VGLUT is an unusual protein that has two independent transport machineries in a single molecule. Multifunctionality is a common feature in all SLC17 transporters. For instance, NPT1 exhibits $\Delta\psi$-driven organic anion transport and Na^+ gradient-driven inorganic phosphate transport activities.[45] VEAT has $\Delta\psi$-driven glutamate and aspartate transport activity when it is present in synaptic vesicles. When VEAT is located in lysosomes, it functions as a H^+/sialic acid cotransporter.[5]

■ BIOENERGETICS OF VESICULAR GLUTAMATE TRANSPORT

The energetics of neurotransmitter transport is a long-standing but still crucial problem. Because the maximal luminal

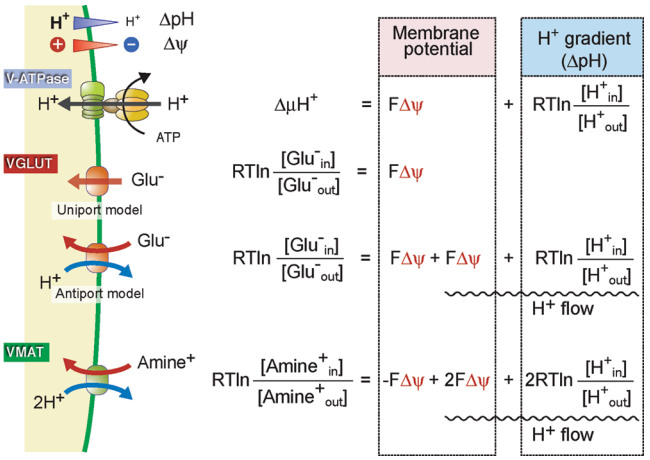

Figure 4. Energetics of glutamate and monoamine transport. V-ATPase pumps H^+ into secretory vesicles and establishes an electrochemical gradient of H^+ ($\Delta\mu H^+$). The $\Delta\mu H^+$ is composed of membrane potential ($\Delta\psi$) and H^+ gradient (ΔpH). VGLUTs concentrate glutamate by using $\Delta\mu H^+$ as the driving force. Energy components of glutamate transport with uniport and H^+ antiport models are shown. For comparison, energy components of monoamine transport by VMAT are shown at the bottom.

concentration of a neurotransmitter is determined by the driving force, the energetics of neurotransmitter transport can influence signal transmission through modification of quantum size. Classical biochemical analyses using secretory vesicles such as chromaffin vesicles and synaptic vesicles have demonstrated that the neurotransmitter uptake level correlates with the intensity of the electrochemical gradient of H^+ ($\Delta\mu H^+$) established by V-ATPase.[46,47]

Because pumping of H^+ across the membrane generates an electrical gradient along with a pH gradient, $\Delta\mu H^+$ is given by

$$\Delta\mu H^+ = F\Delta\psi + RT \ln[H^+_{in}]/[H^+_{out}]$$

where F is the Faraday constant, R is the gas constant, T is the absolute temperature, $[H^+{}_{in}]$ is the luminal H^+ concentration, and $[H^+{}_{out}]$ is the cytoplasmic H^+ concentration (Figure 4). Depending on the coupling ion and substrate charge, the degree of contribution of ΔpH and $\Delta\psi$ to transport is different.

In the case of glutamate transport, glutamate is negatively charged at physiological pH, indicating that an inside positive $\Delta\psi$ is more important than ΔpH. A series of studies showed that the primary driving force of glutamate transport is $\Delta\psi$.[17,38,48] If membrane potential is the sole driving force, the chemical potential for the transport of glutamate across the synaptic vesicle membrane is given by the following equation (Figure 4).

$$RT \ln[Glu^-_{in}]/[Glu^-_{out}] = F\Delta\psi$$

Applying a typical membrane potential of secretory vesicles of ~80 mV to this equation, we calculate the glutamate concentration gradient to be 22.[49,50] This value is in good agreement with a reported concentration gradient of ~10 in synaptic vesicles.[51] This suggests that membrane potential can be responsible for the total uptake of glutamate in synaptic vesicles and that ΔpH is not required for transport. Recent biochemical analyses using purified and reconstituted VGLUT2 also support this idea.[39,40] Reconstituted proteoliposomes containing purified VGLUT2 transport glutamate when an inside positive membrane potential

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is applied, whereas ΔpH fails to drive glutamate transport. Glutamate is an abundant amino acid in the cytoplasm with a concentration of around 10 mM.[41,42] Such high cytoplasmic concentrations of glutamate lead to sufficient luminal concentrations for signal transmission with minimal energy consumption.

However, there are conflicting reports about the contribution of ΔpH to glutamate transport.[17,37,43,44,48] In these cases, VGLUT is assumed to be a glutamate/H^+ antiporter. The movement of H^+ across the membrane adds additional ΔpH and $\Delta\psi$ components to the driving force. Thus, the chemical potential of glutamate is expressed as follows and corresponds to a 15400-fold concentration gradient (Figure 4).

$$RT \ln[Glu^-_{in}]/[Glu^-_{out}] = F\Delta\psi + F\Delta\psi + RT \ln[H^+_{in}]/[H^+_{out}] = 2F\Delta\psi + RT \ln[H^+_{in}]/[H^+_{out}]$$

Clearly, the driving force is too large to concentrate glutamate.[51]

$\Delta\psi$-driven transport is a common feature of all SLC17 transporters.[5,6,45] When VEAT is purified and reconstituted, $\Delta\psi$ stimulates aspartate and glutamate transport whereas ΔpH failed to drive transport. This is also true for NPT1. The recent identification of and kinetic studies of VNUT also speak for $\Delta\psi$-driven anion transport. Under physiological conditions, ATP is primarily in a complex with Mg^{2+} as Mg^{2+}-ATP^{4-}.[52] Kinetic studies of nucleotide transport showed that VNUT can transport ATP together with Mg^{2+} in a $\Delta\psi$-dependent manner (manuscript in preparation). For a divalent anionic type of ATP complex, the concentration gradient can be calculated as follows.

$$RT \ln[ATP_{in}]/[ATP_{out}] = 2F\Delta\psi$$

This gives a 488-fold concentration of ATP when $\Delta\psi$ is 80 mV. The value corresponds to ~200 mM luminal ATP and is on the same order of magnitude as that previously reported.[53] If VNUT transports ATP at the expense of a H^+ counterflow, the concentration gradient of ATP can be calculated as follows.

$$RT \ln[ATP_{in}]/[ATP_{out}] = 3F\Delta\psi + RT \ln[H^+_{in}]/[H^+_{out}]$$

This gives a 340000-fold concentration gradient. Such a high level of accumulation of ATP is not required for signal transmission. Thus, energetically, H^+ counterflow is not required.

Compared to those of VGLUT and VNUT, the energetic situation of SLC18 transporters such as VMAT is totally different. VMAT transports monoamines at the expense of ΔpH and $\Delta\psi$.[46,47,54,55] Detailed kinetic studies showed that VMAT functions as a H^+ antiporter and its stoichiometry is one molecule of monoamine per two protons.[46,54] Thus, VMAT transports cationic monoamines against an inside positive $\Delta\psi$ at the expense of $\Delta\mu H^+$ provided by the counterflow of two protons. The chemical potential of monoamine transport can be expressed as follows (Figure 4):

$$RT \ln[amine^+_{in}]/[amine^+_{out}] = -F\Delta\psi + 2\Delta\psi H^+ = F\Delta\psi + 2RT \ln[H^+_{in}]/[H^+_{out}]$$

Kinetic studies clearly show the linear correlation of monoamine accumulation with this stoichiometry.[47] This means that the major driving force of monoamine transport is ΔpH and that $\Delta\psi$ is less effective. Using a typical $\Delta\psi$ of 80 mV and a ΔpH of 1.5 for secretory vesicles, the maximal concentration gradient achieved by VMAT is calculated to be 22000-fold. The reported concentration gradient of catecholamine across the chromaffin granule membrane is 10000−100000-fold; these values are corroborated by energetic calculations.[47,53,55−58] Again, the energetics of VMAT fully explains the concentration of monoamines in secretory vesicles. After all, the energetics of glutamate and other neurotransmitter transport is the most critical factor for vesicular concentration.

REGULATION OF VGLUT

Control of vesicle energetics and neurotransmitter transporters are important factors in determining the luminal concentration of neurotransmitters and subsequent signal transmission.[40,59] The regulation of ΔpH and $\Delta\psi$ by Cl^- in synaptic vesicles has attracted researchers for many years. The ΔpH and $\Delta\psi$ established by V-ATPase are highly dependent on Cl^- concentration.[38,43,60] Although ΔpH is enhanced by an increasing Cl^- concentration, $\Delta\psi$ is inhibited by Cl^-.[38,39] Thus, at lower Cl^- concentrations, $\Delta\psi$ is a major component of $\Delta\mu H^+$, and at >100 mM Cl^-, ΔpH becomes the sole component of $\Delta\mu H^+$ (Figure 3). The entry of chloride into synaptic vesicles reduces $\Delta\psi$, and hence, V-ATPase transports more H^+ into vesicles, which results in increased ΔpH. However, the molecular mechanism of Cl^- entry is not clear at this time. The CLC-3 Cl^- channel is a candidate for regulating vesicular ΔpH.[1,61] Synaptic vesicles isolated from CLC-3 knockout mice still retain Cl^--dependent acidification and biphasic Cl^- dependence of glutamate transport. Thus, CLC-3 is not responsible for these Cl^- dependencies.[43,62]

When NPT1, the first identified member of the SLC17 family, was expressed in oocytes, a Cl^- current was observed.[63,64] On the basis of the Cl^- current and the biphasic Cl^- dependence of glutamate transport, VGLUT is assumed to be permeable to Cl^-.[43] In this model, entry of Cl^- through VGLUT reduces $\Delta\psi$ and enhances ΔpH at higher Cl^- concentrations. On the other hand, freshly endocytosed vesicles contain high concentrations of Cl^- at low cytoplasmic Cl^- concentrations, Cl^- leaks out from synaptic vesicles, and VGLUT generates an inside positive $\Delta\psi$.[43] In newly endocytosed synaptic vesicles, V-ATPase does not have enough time to establish a $\Delta\mu H^+$; thus, leakage of Cl^- from fresh synaptic vesicles is an important path for supplying driving force.

On the other hand, direct measurement of Cl^- transport using purified VGLUT2 incorporated into liposomes did not show Cl^- transport activity.[40] Also, the difference in kinetics between Cl^--dependent and glutamate-dependent acidifications indicated that Cl^- permeability is not correlated with VGLUT activity.[65] To clarify these reported discrepancies, the Cl^- permeability of VGLUT and the Cl^- regulation of ΔpH need further study.

As an alternative to the Cl^- transport model, the Cl^- activation model was proposed.[40] In this model, VGLUT activity is regulated by direct binding of Cl^- to VGLUT (Figure 3). Interestingly, when Cl^- dependence is measured in VGLUT incorporated into liposomes as the sole protein component, the kinetics of glutamate transport becomes single-phase, activated by a few millimolar Cl^- with no inhibition at higher Cl^- concentrations. This clearly shows that the inhibition of glutamate transport by high Cl^- concentrations observed in synaptic vesicles is not due to the permeation of Cl^- through VGLUT.

Another remarkable feature discovered by using this simple reconstitution system is the strong cooperativity of Cl^- activation. Stimulation of VGLUT activity by few millimolar Cl^- exhibits a Hill coefficient of 3−4, indicating strong cooperativity between Cl^- binding sites. Strong cooperativity means that the activity of VGLUT is strictly regulated. For instance, VGLUT is almost inactive at 2 mM Cl^-, whereas full activity is observed at 5 mM Cl^-. A strong Cl^- dependence is a common feature of SLC17 members, including

VGLUT1, VGLUT3, VNUT, VEAT, and NPT1.[5,6,39,40,45] Such Cl^- regulation is specific to SLC17 and is not observed in VMAT.[40]

In neurons, the Cl^- concentration is maintained at 10−30 mM by the KCC2 and NKCC Cl^- transporters.[66] Thus, under normal conditions, VGLUTs will be fully active. Rather unexpectedly, stimulation of VGLUT by Cl^- is competitively inhibited by keto acids and related compounds such as pyruvate and acetoacetate.[40] These keto acids are metabolic intermediates of glycolysis and amino acid and fatty acid metabolism. These results show the functional interrelationship between metabolic state and glutamatergic signal transmission. Among these compounds, acetoacetate and 5-hydroxybutyrate are well-known as ketone bodies produced with a ketogenic diet or fasting. Acetoacetate reduces the quantal size of quantum glutamate release in mouse hippocampal slices and 4-aminopyridine-evoked seizure. Over the years, fasting and a ketogenic diet have been used to control epilepsy, but the molecular mechanism underlying these treatments is not understood.[67] Inhibition of VGLUT activity by ketone bodies showed why a ketogenic diet is effective in the control of epilepsy. In addition to ketone bodies, VGLUT is inhibited by α-keto acids produced in maple syrup urine disease and phenylketonuria disease. This suggests that glutamatergic signaling and these genetic diseases may be also interrelated.

In addition to keto acids and ketone bodies, many compounds are reported to inhibit VGLUT.[68−74] Those compounds include amino acids, fatty acids, azodyes, quinolines, and alkaloids. Like kynurenic acid, quinoline-related compounds produced by tryptophan metabolism are well-known to exhibit convulsant and/or anticonvulsant activity.[70,71] These compounds are reported to inhibit vesicular uptake of glutamate. It is possible that kynurenic acid works as another intrinsic regulator of glutamatergic signaling.[70] Arachidonic acid and polyunsaturated fatty acids are also reported to inhibit vesicular glutamate transport.[74] These compounds are released under pathological conditions such as ischemia and seizures and inhibit vesicular uptake of glutamate and GABA through their proton ionophore activity. Although these reports were not tested with the purified transporter, there may be many intrinsic compounds that regulate VGLUT activity.

PERSPECTIVE

Recent biochemical analyses of VGLUT demonstrate that Cl^- plays a pivotal role in glutamatergic signaling. Modulation of Cl^- effects by metabolic intermediates shows the interdependency of metabolic state and higher-order brain function. The possibility of controlling epilepsy through a ketogenic diet tells us that VGLUT is a potential pharmacological target for the treatment of epilepsy and other neuropathic diseases.

We propose that Cl^- is a novel regulator for signal transmission and that it has a fundamental role in the modulation of excitatory signal transmission. However, the molecular mechanism underlying Cl^- regulation of VGLUT and $\Delta\mu H^+$ remains to be clarified. In addition, there are significant discrepancies between reports in the literature. Revisiting the basic biochemistry of secretory vesicles with new technology is required to solve unanswered questions.

AUTHOR INFORMATION

Corresponding Author

*Department of Membrane Biochemistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8530, Japan. Telephone: 86-251-7935. Fax: 86-251-7933. E-mail: omote@pheasant.pharm.okayama-u.ac.jp.

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ABBREVIATIONS

VGLUT, vesicular glutamate transporter; VNUT, vesicular nucleotide transporter; VEAT, vesicular excitatory amino acid transporter; VMAT, vesicular monoamine transporter; VGAT, vesicular GABA transporter; VIAAT, vesicular inhibitory amino acid transporter; VAChT, vesicular acetylcholine transporter; GABA, γ-aminobutyric acid; $\Delta\mu H^+$, electrochemical potential of H^+; $\Delta\psi$, membrane potential.

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