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Vesicular glutamate transporters (VGLUTs): The three musketeers of glutamatergic system

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Review

Abstract. Glutamate is the predominant excitatory neurotransmitter in the central nervous system (CNS) and glutamatergic transmission is critical for controlling neuronal activity. Glutamate is stored in synaptic vesicles and released upon stimulation. The homeostasis of glutamatergic system is maintained by a set of transporters present in plasma membrane and in the membrane of synaptic vesicles. The family of vesicular glutamate transporters in mammals is comprised of three highly homologous proteins: VGLUT1-3. The expression of particular VGLUTs is largely complementary with limited overlap and so far they are most specific markers for neurons that use glutamate as neurotransmitter. VGLUTs are regulated developmentally and determine functionally distinct populations of glutamatergic neurons. Controlling the activity of these proteins could potentially modulate the efficiency of excitatory neurotransmission. This review summarizes the recent knowledge concerning molecular and functional characteristic of vesicular glutamate transporters, their development, contribution to synaptic plasticity and their involvement in pathology of the nervous system.

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Key words: vesicular glutamate transporter, VGLUTs, brain, glutamate transport

INTRODUCTION

Glutamate was discovered by Kikunae Ikeda, a professor of Tokyo Imperial University in 1908, while looking for the flavor common to foods like cheese, meat, and mushrooms. He was able to extract the acid glutamate from seaweed, but it took about five decades for scientists to discover that this substance can excite the brain tissue (Chiosa and Gane 1956, Curtis et al. 1960), and two decades more to accept it as the main excitatory neurotransmitter in the central nervous system (Fonnum 1984). L-glutamate is a common amino acid playing a key role in cellular metabolism. However, in the CNS it is the major excitatory neurotransmitter and is used by as much as half of all neurons in the brain. Glutamate can be produced intramitochondrially from glutamine by the action of phosphate-activated glutaminase and then undergoes a transamination catalyzed by the mitochondrial isoform of aspartate aminotransferase. The resulting α-ketoglutarate is translocated out of the mitochondria and transaminated in the cytoplasm by the cytoplasmic isoform of aspartate aminotransferase. Alternatively, glutamate may be formed from α-ketoglutarate and alanine catalyzed by alanine aminotransferase. The cytoplasmic glutamate is transported into vesicles by the vesicular glutamate transporters (VGLUTs). After the glutamate has been released to the synaptic cleft, it can be either inactivated by enzymatic degradation or transported back to the neuron or into the glial cells by active transport (Fig. 1). According to the structure and site of action, glutamate transporters, as other neurotransmitter transporters, can be divided into two superfamilies: the plasma membrane transporters (EAATs) and the vesicular transporters (VGLUTs).

There are some substantial differences between the two types of transporters. First, in contrast to plasma membrane glutamate uptake (Amara and Kuhar 1993, Kanner 1993), the accumulation of glutamate in synaptic vesicles does not rely on a Na^+ electrochemical gradient. Second, vesicular glutamate transport has a substantially lower affinity for glutamate (K_m of ~1 mM) than the plasma membrane excitatory amino acid transporters (K_m of ~4–40 μM) (Shigeri et al. 2004). Third, plasma membrane glutamate transporters recognize both aspartate and glutamate as substrates, whereas vesicular glutamate transporters do not recognize aspartate (Maycox et al. 1990).

VGLUTs are dependent on a proton gradient that they create by hydrolyzing adenosine triphosphate (ATP) with V-type H^+-ATPase. This enables the flow of H^+ into the interior of the synaptic vesicle making it more acidic and generating a pH gradient across the

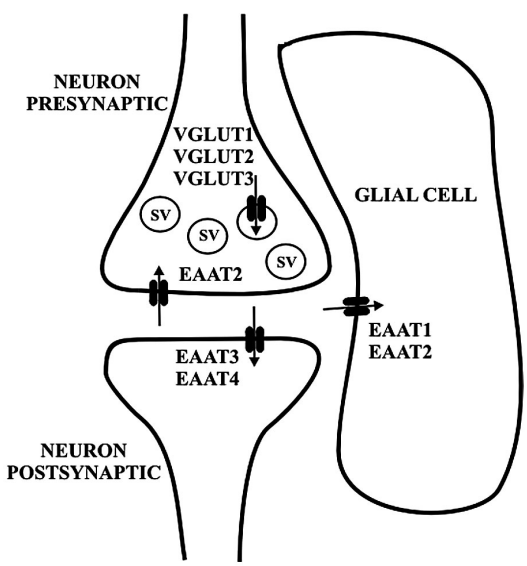

Fig. 1. Distribution of plasma membrane and vesicular glutamate transporters in different cellular compartments. (EAAT1-4) Excitatory amino acid transporters; (SV) synaptic vesicles; (VGLUT1-3) vesicular glutamate transporters. Arrows indicate the direction of glutamate transport.

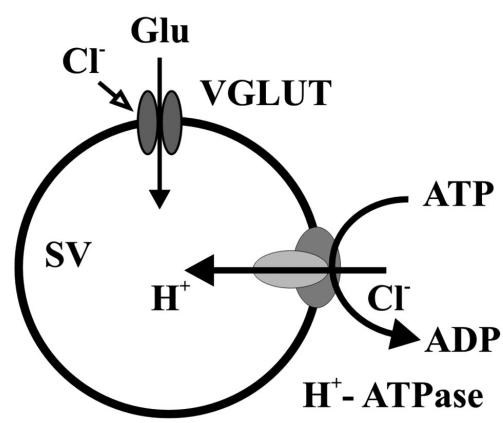

Fig. 2. Mechanism of glutamate transport into the synaptic vesicle (SV). Vesicular glutamate transporters use proton electrochemical gradient generated by vacuolar ATPase to carry the glutamate anion (Glu) into the interior of synaptic vesicles. Low concentration of chloride ensures the best VGLUTs efficiency.

vesicle membrane (Fig. 2). The second consequence of the proton influx is that vesicle interior becomes more positive creating a corresponding membrane potential, thus forming electrochemical proton gradient. Moreover, for optimal vesicular glutamate transport the presence of low concentration of chloride (1–5 mM) or bromide is needed (Naito and Ueda 1985). VGLUTs have a strong affinity for substrate recognition and they prefer L-glutamate to the D-form (Moriyama and Yamamoto 1995). The accumulation of the neurotransmitters into storage vesicles ensure the quantal character of neurotransmission and control the gradient of neurotransmitter concentration across the plasma membrane. The effective transport systems also protect neurons from accumulation of neurotransmitter and the neurotoxic effect of glutamate.

A BIT OF HISTORY

Although the glutamate has been known a neurotransmitter for almost 25 years, its transport into the synaptic vesicles was long undiscovered. In 1994 Ni and coworkers discovered a gene upregulated in a primary culture of rat cerebellar granule cells after treatment with N-methyl-D-aspartate (NMDA). The protein coded by this gene was able to transport inorganic phosphate in a Na^+-dependent manner. In the rat brain the expression of this gene was restricted to a particular subset of neurons. For these reasons it was named brain – specific Na^+-dependent inorganic phosphate co-transporter (BNPI) (Ni et al. 1994). However, after six years of investigation it turned out that surprisingly BNPI was localized in presynaptic terminals where associated with synaptic vesicles (Bellocchio et al. 1998). Additionally, it has been shown that mutations in eat-4, which is a BNPI homolog in *C. elegans*, caused deficits in glutamatergic transmission (Lee et al. 1999). Finally in 2000, two papers appeared that independently confirmed the role of BNPI in glutamate transport into the synaptic vesicles and consequently BNPI was renamed vesicular glutamate transporter VGLUT1 (Bellocchio et al. 2000, Takamori et al. 2000). Simultaneously, Aihara found the protein highly homologous to VGLUT1. It was DNPI, a differentiation – associated Na^+-dependent inorganic phosphate transporter that was upregulated during the differentiation of rat pancreatic tumor cell line AR42J into neuron-like cells (Aihara et al. 2000). DNPI had 82% amino acid homology with VGLUT1 and numerous experimenters established its expression in glutamatergic neurons and its role in vesicular glutamate transport, thus calling it VGLUT2 (Fujiama et al. 2001, Herzog et al. 2001, Sakata-Haga et al. 2001, Takamori et al. 2001). The two isoforms appeared to account for the release of glutamate by all known glutamatergic neurons, but there was evidence that a number of dopaminergic and serotonergic neurons as well as astrocytes might also release glutamate (Araque et al. 2000, Bezzi et al. 1998, Johnson 1994, Newman and Zahs 1998, Sulzer et al. 1998). These cells however, failed to express any of the known VGLUTs. That is why scientists started to look for another glutamate transporter and in 2002 the third member of the vesicular glutamate transporter family – VGLUT3 was identified (Gras et al. 2002, Schafer et al. 2002).

All three isoforms are highly homologous. The membrane spanning domains of VGLUTs have almost 90% homology, whereas the N- and C-terminal regions have little homology and contribute to functional differences (Fig. 3). It seems that the genes for these proteins are evolutionary conserved, as multiple VGLUT isoforms have been identified in many organisms from different branches of philogenetic tree, i.e., *Drosophila* (Daniels et al. 2004), zebrafish (Higashijima et al. 2004), or frog (Gleason et al.

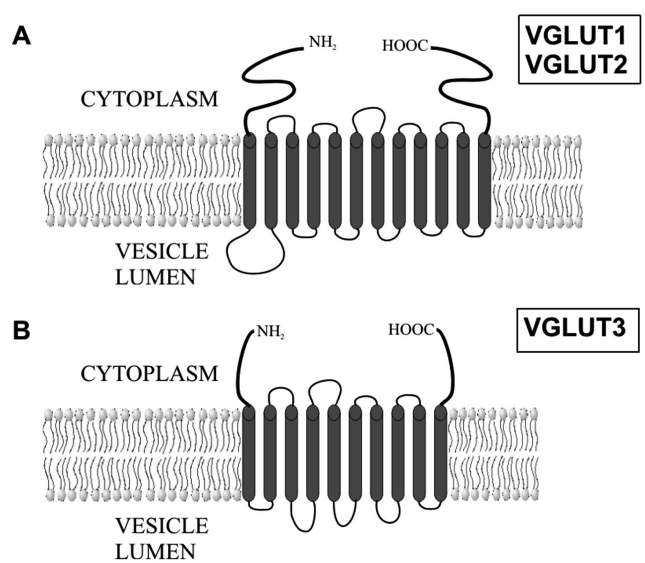

Fig. 3. Predicted secondary structure of VGLUTs. Putative models propose twelve transmembrane segments for VGLUT1 and VGLUT2 (A) and ten domains for VGLUT3 (B) with both terminals facing the cytoplasm.

Table I

Comparison of molecular and biochemical characteristics of VGLUTs

	VGLUT1	VGLUT2	VGLUT3
Atomic mass (kDa)	61	64.4	64.7
Number of amino acids	560	582	589
Number of transmembrane domains	6–12	12	10
C- and N-terminal domains	Intracellular	Intracellular	Intracellular
K_m (mmol/L)	3.4	0.8	0.52
V_{max} (pmol/mg/min)	500	190	20.3
Phosphorylation	Tyrosine kinase, PKC, CaMKII	?	?
Inhibitors	H^+ ionophore, Evans blue, kynurenic acid, bromocriptine	Evans blue, kynurenic acid, bromocriptine	H^+ ionophore, kynurenic acid, bromocriptine

2003). It also seems that transport properties of the VGLUTs are similar (Bellocchio et al. 2000, Fremeau et al. 2001), Takamori et al. 2000 (see Table I). Why then have three isoforms emerged in mammals? Difference in cellular and regional distribution of these transporters may, in part answer that question.

VGLUTS IN CNS

VGLUT1 and VGLUT2

These two isoforms are expressed mainly in glutamatergic neurons and their expression in CNS seems to be largely complementary with only a limited overlap (Fig. 4, see also Table II). VGLUT1 is localized mainly in the neocortex (layers I–III), entorhinal and piriform cortex, hippocampus, amygdala and subiculum. VGLUT2 can be observed in olfactory bulb, layer IV of the cerebral cortex, granular layer of the dentate gyrus, thalamus, hypothalamus and in the brain stem (Fremeau et al. 2001, Fujiyama et al. 2001, Herzog et al. 2001, Kaneko and Fujiyama 2002, Kaneko et al. 2002). In the cerebellum, VGLUT1 is expressed in parallel fibers and VGLUT2 in climbing fibers and in Purkinje cell dendrites (Fremeau et al. 2001, Hisano et al. 2002). Such a complementary distribution has inspired scientists to make an attempt to functionally categorize glutamatergic neurons on the basis of the VGLUT isoform expressed. They have proposed that VGLUT1 is present at synapses with low release probability, which are known to exhibit long-term potentiation (LTP), whereas VGLUT2 is expressed in synapses that exhibit high release probability and long-term depression (LTD) (Fremeau et al. 2001, Varoqui et al. 2002). It was in line with the data that had revealed the highest level of VGLUT2 detected early in the development and that high release probability promotes sur-

Table II

Intensity of VGLUTs expression in different regions of the brain

	VGLUT1	VGLUT2	VGLUT3
Neocortex	♦♦♦	♦♦	♦
Striatum	♦♦♦	♦♦	♦♦
Nucleus Accumbens	♦♦	♦♦♦	♦
Piriform cortex	♦♦♦	♦	♦
Hippocampus	♦♦♦	♦	♦♦
Thalamus	♦♦♦	♦♦	♦
Hypothalamus	♦	♦♦♦	♦
Cerebellum	♦♦♦	♦♦	♦

(♦♦♦) strong immunoreactivity; (♦♦) medium immunoreactivity; (♦) weak immunoreactivity (Herzog et al. 2004, Hisano 2003)

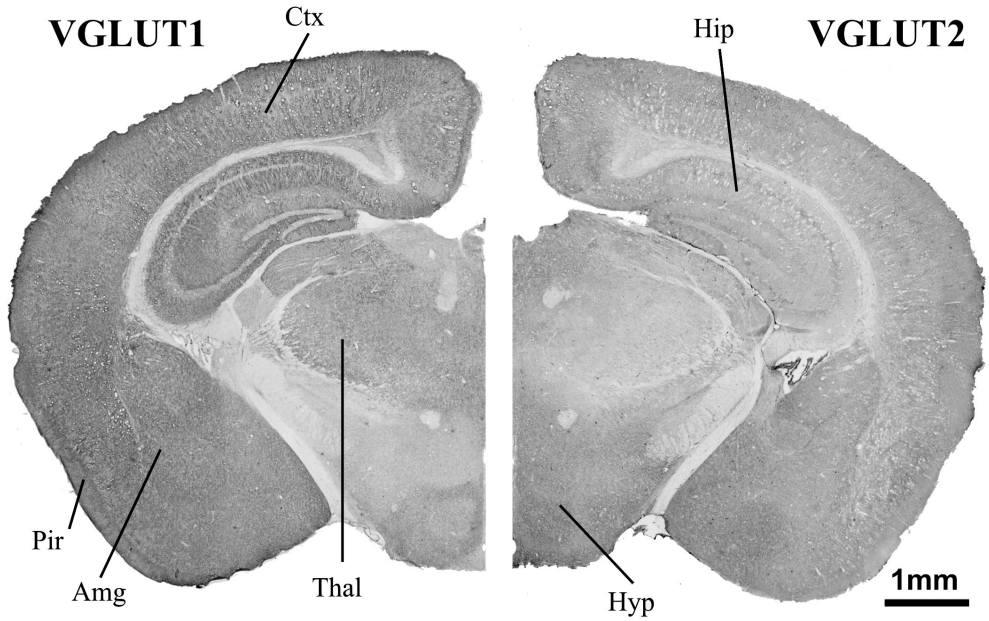

Fig. 4. Complementary distribution of VGLUT1 and VGLUT2 in the mouse brain. Peroxidase staining of coronal sections stained with anti VGLUT1 rabbit polyclonal antibody (Synaptic System) and anti VGLUT2 monoclonal antibody (Chemicon). Structures: (Amg) amygdala; (Ctx) cortex; (Hip) hippocampus; (Hyp) hypothalamus; (Pir) piriform cortex; (Thal) thalamus.

vival and maturation of synapses (Komuro and Rakic 1993, Marini et al. 1998). Nowadays we know that such classification is not so simple, since some neurons may temporarily in the development or even permanently co-express both isoforms of VGLUTs. Such co-expression was shown in the hippocampus (Herzog et al. 2006), neocortex (Liguz-Lecznar and Skangiel-Kramska 2007, Nakamura et al. 2005), spinal cord (Persson et al. 2006) and in the cerebellum (Boulland et al. 2004), where a developmental switch from one isoform to another can be seen (Miyazaki et al. 2003). It was proposed that in these neurons VGLUTs can be segregated to different synapse populations (segregation model) or colocalized in the same synapse. If the last is true, they can be distributed in distinct synaptic vesicles (commingling model) or colocalize in the same vesicle (colocalization model). Some of these models have been experimentally confirmed. Fremau and coauthors (2004a) have shown that in VGLUT1 knockouts the amplitude of mEPSC recorded in brain slices is unaltered. Moreover applying a NMDA receptor channel blocker – MK801 did not slow the rate of blocking kinetics, what corroborates with the segregation model (Fremau et al. 2004a). Two other groups however, have provided evidence for the existence of colocalization model. Wojcik and coworkers (2004) worked with VGLUT1$^{-/-}$ autaptic culture (where neurons make synapses between axons and cell bodies from which they arise) from hippocampal neurons and were able to detect small EPSCs in the majority of cells due to the exocytosis of partially filled vesicles expressing VGLUT2 (Wojcik et al. 2004). Herzog and coauthors (2006) used synaptic vesicles immunoisolated with beads coated with antibodies against VGLUT1 or VGLUT2 and have shown that both transporters were localized in the same vesicles (Herzog et al. 2006). The two models presented do not disprove each other. Rather we can expect that in some synapses one type of transporter will be expressed exclusively and in others, both isoforms will be present in the same synaptic vesicles. However, in the adult nervous system, VGLUT2 is generally considered a marker of glutamatergic neurons of thalamic origin, whereas VGLUT1 – a marker of cortical terminals (Fujiyama et al. 2004, 2006, Hur and Zaborszky 2005, Kubota et al. 2007). It has been shown moreover that either isoform of VGLUTs is associated with different regulatory proteins. Their dependence on chloride ions is regulated by G proteins with different subunits profiles (Ahnert-Hilger et al. 2003, Brunk et al. 2006, Pahner et al.

2003) and each isoform is associated with distinct endophilins, the proteins regulating synaptic vesicle formation and trafficking (De Gois et al. 2006).

Unexpectedly, VGLUT1 and VGLUT2 mRNA and protein were found in some isolated dopaminergic neurons (Dal Bo et al. 2004, Kawano et al. 2006, Trudeau 2004), GABAergic and cholinergic neurons of cerebellum and striatum (Danik et al. 2005). It has been proposed that corelease of glutamate with GABA may contribute to inhibitory synaptic plasticity and that corelease of glutamate together with acetylcholine might activate silent synapses. Since silent synapses do not contain AMPA receptors, which are necessary for activation of the NMDA receptor and generation of the action potential, glutamate released together with acetylcholine could help to depolarize postsynaptic membrane without contribution of AMPA receptor (Duguid and Smart 2004, Seal and Edwards 2006).

VGLUT3

The third vesicular glutamate transporter is localized in a limited number of glutamatergic neurons in multiple brain regions: neocortex, hippocampus, olfactory bulb, hypothalamus, substantia nigra, raphe nuclei (Fremeau et al. 2002, Gras et al. 2002, Herzog et al. 2004, Schafer 2002). Additionally, VGLUT3 is expressed in a population of symmetrical synapses. It has been found in hippocampal and cortical GABAergic neurons (Herzog et al. 2004), cholinergic neurons in the striatum and serotonergic neurons in the raphe nuclei (Gras et al. 2002, Schafer et al. 2002). These observations support the idea that glutamate can be stored and maybe even co-released together with another classical transmitter, modulating neuronal transmission. The distribution of VGLUT3 is quite widespread, but simultaneously, very restricted to particular populations of neurons. It is the only vesicular glutamate transporter present in the dendrites of striatal neurons (Fremeau et al. 2002). In some brain regions, like septum, amygdala, hypothalamus, substantia nigra or entorhinal cortex, VGLUT3-immunopositive terminals can be found, although no VGLUT3 mRNA can be detected. Thus in those regions VGLUT3 appears to be expressed by innervating them long projecting neurons (Herzog et al. 2004). Interestingly, unlike VGLUT1 and VGLUT2, VGLUT3 can be found not only in presynaptic terminals, but also, more rarely, postsynaptically on dendrites, and in cell bodies (Fremeau et al. 2002, 2004b). This can testify to VGLUT3 involvement in somatodendritic release of glutamate or a different (other than transporting) role of VGLUT3 in this neuronal compartment (Herzog et al. 2004). Unlike VGLUT1 and VGLUT2, which can be seen in the same axonal terminals, VGLUT3 does not colocalize with other VGLUT isoforms (Boulland et al. 2004). Thus, VGLUT3 delineates a new subset of excitatory fibers, suggesting a new role for glutamatergic signaling.

BEYOND THE BRAIN

All VGLUTs can be found in the retina, spinal cord, peripheral nervous system (PNS) as well as in non-neuronal cells. VGLUTs are localized in the dorsal root ganglia, where they are involved in nociception (Oliviera et al. 2003), in the trigeminal ganglion (Li et al. 2003) and in enteric neurons being the primary afferent neurotransmitter that transfers information from the mucosa to the enteric plexuses and brain (Tong et al. 2001). In the retina the three isoforms have complementary distribution. VGLUT1 is expressed in the photoreceptors and in bipolar cells and VGLUT2 in ganglion cells (Mimura et al. 2002). VGLUT3 can be found in a subset of amacrine cells (Fremeau et al. 2002). Outside the nervous system the vesicular glutamate transporters can be found in the glutamate-secreting non-neuronal cells of different organs, such as the pineal gland (Morimoto et al. 2003); within islets of Langerhans (Hayashi et al. 2003), in the intestine and stomach (Hayashi et al. 2003), bone (Hinoi et al. 2004) and testes (Redecker et al. 2003). Glutamate is also stored and released as a neurotransmitter in terminals of several pulmonary (sensory) nerve fiber populations where is involved in the peripheral transmission of sensory stimuli from the lungs (Brouns et al. 2004). It has been proposed that this peripheral glutamatergic system may use the glutamate as an intercellular messenger to communicate with neighboring cells and that the mode of glutamate signal transmission is diverse in nature in CNS, PNS and non-neuronal cells (Moriyama and Yamamoto 2004).

DEVELOPMENT

The expression of the vesicular glutamate transporters is age-dependent and developmental regulation of particular VGLUTs is different in distinct brain regions. Axon terminals containing VGLUT2 mature earlier than VGLUT1- and VGLUT3-loaded axons and

its expression at birth is relatively high (Boulland et al. 2004). Generally, expression of VGLUT1 is known to increase during postnatal development in most brain regions (Minelli et al. 2003, Nakamura et al. 2005). However, a detailed analysis of VGLUT1 expression in the barrel cortex revealed significant decrease in VGLUT1 immunoreactivity after P14 (Liguz-Lecznar and Skangiel-Kramska 2007). VGLUT2 immunoreactivity also tends to increase with age in the cortex (Liguz-Lecznar and Skangiel-Kramska 2007), but in the cerebellum after first postnatal week, it declines by 14-fold (Boulland et al. 2004). Similar changes can be seen with VGLUT3. There is a progressive increase in the level of expression of VGLUT3 in the cortex, while in the cerebellum it declines five-fold from P7 to adulthood (Boulland et al. 2004). Gras and coworkers (2005) have described a biphasic profile of VGLUT3 developmental expression, measured in total extracts from rat brain, with the first peak around P10 and the second one in adult animals (Gras et al. 2005). These differences may be due to regional peculiarity, but can also come out of the developmental time points analyzed in particular experiments. It has been shown that in some regions individual VGLUTs are expressed only transiently and in others, one VGLUT isoform can be replaced by another one. A transient expression of VGLUT3 can be observed in the auditory brainstem between P0 and P12 and it is probably involved in the maturation of the glycynergic projection in this region (Blaesse et al. 2005). During development, in the mouse cerebellum, in pyramidal cells of the hippocampus and in the subpopulation of cortical neurons, there is a switch from VGLUT2 to the VGLUT1 isoform which is associated with physiological maturation of neuronal connections within particular structures (Miyazaki et al. 2003). The subtype switching phenomenon is connected to co-expression of the two VGLUT isoforms in the same nerve terminals. Such colocalization can be seen transiently in early postnatal life (Herzog et al. 2006, Nakamura et al. 2005) or even permanently until the adulthood (Liguz-Lecznar and Skangiel-Kramska 2007) and is thought to be involved in the period of high neuronal plasticity. Since it has been shown that VGLUT1 as well as VGLUT2 level control quantal size and efficacy of neurotransmission (Moechars et al. 2006, Wojcik et al. 2004) and that both isoforms can colocalize in the same synaptic vesicle (Herzog et al. 2006), neurons expressing both isoforms may be more effective and may differentially respond to regulatory inputs during the brain development (Herzog et al. 2004).

PLASTICITY

Experiments with overexpression of VGLUT1 and knocking out VGLUT1 and VGLUT2 genes brought contradictory results. Wojcik and coworkers (2004) working with cultured hippocampal neurons found that the amount of VGLUT protein on the synaptic vesicle determines the amount of glutamate released, but it is not sufficient to predetermine the release probability (Wojcik et al. 2004). The experiments of Wilson and coworkers provided convincing patch-clamp evidence that overexpression of VGLUT1 increased EPSC amplitude and decreased the failure rate of evoked transmission (Wilson et al. 2005). However, when Fremeau and coworkers (2004a) have used a brain slice preparation, their results were contradictory. They found that the expression level of VGLUT doesn't determine the quantal size of glutamate; instead they found that synapses of hippocampal striatum radiatum containing VGLUT1 and VGLUT2 respond differentially to repetitive stimulation, suggesting differences in short-term plasticity (Fremeau et al. 2004a). Thus, the role of individual VGLUTs in determining the quantal size and their involvement in short-term plasticity remains to be clarified. It has been demonstrated that VGLUT1 and VGLUT2 are capable of bidirectional and opposite regulation in response to changes in neuronal activity, thereby contributing to homeostatic plasticity (Erickson et al. 2006). De Gois and others (2005) found that hyperexcitation with $GABA_A$ receptor antagonist – bicuculline resulted in downregulation of VGLUT1 and upregulation of VGLUT2 in primary neocortical cell culture. On the contrary, blocking activity with sodium channel blocker – tetrodotoxin (TTX) increased VGLUT1 and decreased VGLUT2 expression. Such homeostatic scaling of VGLUTs would result in changes of the vesicular loading capacity and regulating the quantal size of glutamate release.

The presence of VGLUT3 in nonglutamatergic neurons seems to contribute to depolarization-induced potentiation of inhibition (DPI) – a newly identified form of inhibitory synaptic plasticity which occurs in cerebellar Purkinje cells. It has been shown that glutamate released from Purkinje cells can act presynaptically via presynaptic NMDA receptor, increasing cal-

cium release from intracellular stores and thereby enhancing the release of GABA (Duguid and Smart 2004, Seal and Edwards 2006).

Moreover, eat-4, a BNPI homolog in *C. elegans*, is involved in habituation to a mechanical tap and may play also a role in dishabituation, which would suggest a role in long-term memory and learning processes (Rankin and Wicks 2000, Rose et al. 2002).

PATHOLOGY

It is well established that increased amounts of extracellular glutamate lead to neurotoxicity (Choi et al. 1987) and in that context the role of plasma membrane glutamate transporters in different animal models of neurological diseases has been broadly investigated. GLUTs are involved in Alzheimer's disease (Masliah 2000), Parkinson's disease (Lievens et al. 2001), Huntington's disease (Behrens et al. 2002), epilepsy (Sepkuty et al. 2002), and cerebral stroke (Maragakis and Rothstein 2004). Since the expression of vesicular glutamate transporters may influence the amount of glutamate released, it could be altered in different pathological processes with the possibility that they can be a target of therapeutic strategies. It has been shown that expression of VGLUT1 was decreased in the hippocampus and dorsolateral prefrontal cortex in brains of schizophrenic patients whereas VGLUT2 expression in the thalamus was upregulated (Eastwood and Harrison 2005, Smith et al. 2001). Reynolds and Harte have found that there is an association between VGLUT1 density and a genetic risk factor for schizophrenia. In their experiments a single polymorphism in neuregulin1, identified as a risk factor for this disease, was associated with lower density of VGLUT1 in human *post-mortem* striatal and hippocampal tissue (Reynolds and Harte 2007, Stefansson et al. 2002). Alterations in VGLUT1 and VGLUT2 expression have also been found in the brain of patients with Parkinson disease. Their levels were increased in Parkinsonian putamen but VGLUT1 expression decreased in prefrontal and temporal cortex of examined patients. These alterations can account for the motor and cognitive disorders observed in Parkinson's disease (Kashani et al. 2007).

Moreover, it turned out that VGLUT1 can be regarded as a marker for antidepressant activity. Moutsimilli and colleagues (2005) have observed a selective increase in VGLUT1 mRNA and protein expression in the cerebral cortex and hippocampus after treatment with antidepressants (fluoxetine, desipramine). Similar results were observed with typical antipsychotic haloperidol and the mood stabilizer lithium. Such plastic adaptation of the glutamate transporting system can represent a common functional endpoint of many antidepressants (Moutsimilli et al. 2005).

CONCLUSIONS

Vesicular glutamate transporters (VGLUTs) are responsible for transport of glutamate into the synaptic vesicles. Three isoforms of VGLUTs (VGLUT1-3) have been identified in mammals. VGLUT1 and VGLUT2 have largely complementary distribution in the CNS with limited overlap and they are considered to be markers of excitatory terminals. VGLUT3 is less abundant in the CNS and often colocalizes with markers of non-glutamatergic neurons. All VGLUTs are developmentally regulated and their expression changes differentially depending on the brain structure. The fact that VGLUTs expression determines the quantal size, establishes the opportunity of modulation glutamatergic transmission and thereby influencing on the synaptic plasticity, namely homeostatic scaling and DPI. There is some evidence of VGLUTs alterations in neurological disorders and therefore functional modulation of these proteins may lead to establishment of new therapeutic procedures in various neurological diseases.

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