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Glutamatergic Chemical Transmission: Look! Here, There, and Anywhere

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Vesicular glutamate transporter (VGLUT) is responsible for the active transport of L-glutamate in synaptic vesicles and thus plays an essential role in the glutamatergic chemical transmission in the central nervous system. VGLUT comprises three isoforms, VGLUT1, 2, and 3, and is a potential marker for the glutamatergic phenotype. Recent studies indicated that VGLUT is also expressed in non-neuronal cells, and localized with various organelles such as synaptic-like microvesicles in the pineal gland, and hormone-containing secretory granules in endocrine cells. L-Glutamate is stored in these organelles, secreted upon various forms of stimulation, and then acts as a paracrine-like modulator. Thus, VGLUTs highlight a novel framework of glutamatergic signaling and reveal its diverse modes of action.

Key words: acrosome, BNPI, chemical transmission, DNPI, L-glutamate, osteoblast, secretory granule, synaptic-like microvesicle, synaptic vesicle, vesicular glutamate transporter, VGLUT.

Abbreviation: BNPI, brain-specific Na^+-dependent inorganic phosphate transporter; DNPI, differentiation-associated Na^+-dependent inorganic phosphate transporter; SLMVs, synaptic-like microvesicles; VGLUT, vesicular glutamate transporter.

L-Glutamate is the major excitatory neurotransmitter in the central nervous system, and plays important roles in many neuronal processes such as fast synaptic transmission and neuronal plasticity. To use L-glutamate as an intercellular signaling molecule, neuronal cells develop a glutamatergic system at glutamatergic synapses, which comprises the output, input, and termination of glutamate signaling (Fig. 1) (*1*). As to signal input, various kinds of glutamate receptors have been identified and characterized. Na^+-dependent glutamate transporters at the plasma membrane are responsible for the signal termination through sequestration of L-glutamate from the synaptic cleft. The signal output systems comprise vesicular storage and subsequent exocytosis of L-glutamate. L-Glutamate is derived from L-glutamine through enzymatic conversion involving phosphate-activated transaminase in neurons, and stored in synaptic vesicles in nerve terminals. Upon stimulation of neurons, synaptic vesicles fuse with the plasma membrane followed by the release of internal L-glutamate into synaptic cleft. Subsequently, synaptic vesicles are retrieved and accumulate L-glutamate again during recycling so as to respond to the next stimulation. Vesicular glutamate transporter (VGLUT) is responsible for the vesicular storage of L-glutamate, and plays an essential role in glutamatergic signaling (*1–3*). Nobody has succeeded in identifying the VGLUT moiety, and thus VGLUT was the missing ring in glutamatergic signal transmission for a long time. In the last year of 20th century, however, two groups independently identified the VGLUT moiety (*4*, *5*). The findings opened a new era of glutamatergic signal transmission. In this article, we give a brief history on VGLUTs and present some unexpected findings as to glutamatergic signal transmission. We discuss how VGLUTs are useful for studying glutamatergic signal transmission.

Identification of VGLUT

Like other neurotransmitters such as acetylcholine and monoamines, L-glutamate is stored in synaptic vesicles before exocytosis (*2*). Vesicular glutamate transporter (VGLUT) is responsible for the storage of L-glutamate (*1–5*). L-Glutamate uptake activity was identified for the first time as the ATP-dependent, proton conductor-sensitive one in synaptic vesicles of rat brain (*6*). Essentially the same activity has been observed in highly purified synapsin I-associated synaptic vesicles (*7*) and synaptic vesicles from various animal species (*8*). Biochemical and bioenergetical analysis has indicated that the glutamate uptake is driven by an electrochemical gradient of protons across the membrane, which is established by vacuolar-type proton ATPase (*9*). Unlike other vesicular neurotransmitter transporters, VGLUT prefers membrane potential differences (positive inside) to pH differences as a driving force (*9*, *10*). The vacuolar-type proton ATPase-coupled L-glutamate uptake activity was solubilized and reconstituted into proteoliposome (*9*). Later, the glutamate uptake activity could be incorporated into liposomes with bacteriorhodopsin (*10*). The proteoliposomes exhibited light-driven L-glutamate uptake, since bacteriorhodopsin forms an electrochemical gradient of protons across the membranes (*10*). Cations

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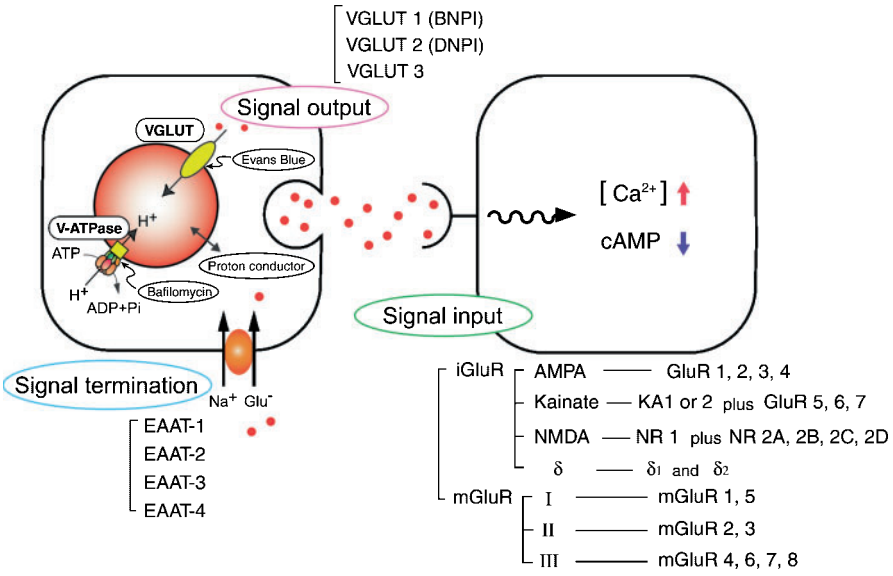

Fig. 1. **The glutamatergic systems comprise output (vesicular storage and exocytosis), input (glutamate receptors), and termination (plasma membrane-type glutamate transporter) components.** L-Glutamate is stored in secretory vesicles and secreted into the extracellular space to transmit glutamate signals. VGLUTs are responsible for vesicular storage, and thus are markers for glutamate signal appearance.

such as Na^+ or K^+ are not necessary for the uptake but a low concentration of Cl^- or Br^- is required for the activity (*9*, *11*, *12*). The requirement of Cl^- is blocked by 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), a stilbene derivative that inhibits anion channels, I^- or SCN^-, suggesting the presence of anion binding site(s) that regulate the transport activity (*11*, *13*). VGLUT shows a strict substrate recognition ability, and recognizes L-glutamate and a few cyclic glutamate analogues such as 1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) and 1-aminocyclohexane-*trans*-1,3-dicarboxylic acid as substrates (*11*). VGLUT prefers L-glutamate as a substrate to the D-form (*11*). D,L-Aspartate is not a substrate for VGLUTs (*9*, *11*, *12*). Evans blue, a dye that contains a glutamate moiety in its structure, and its analogues are known as competitive inhibitors (*14*, *15*).

Many attempts have been made to identify the VGLUT, but were unsuccessful. Identification of the VGLUT moiety occurred in an unexpected field. Na^+-dependent inorganic phosphate cotransporter is responsible for inorganic phosphate homeostasis through its re-absorption in the glomerular filtrate via brush-border epithelial cells in the proximal tubules of the kidney (*16*). A cDNA-encoded homologue of Na^+-dependent inorganic phosphate transporter exhibiting ~32 % overall identity has been cloned from cultured cerebellar granule neurons, its expression being stimulated on incubation with a subtoxic concentration of *N*-methyl-D-aspartate, and designated as brain-specific Na^+-dependent inorganic phosphate transporter (BNPI) (*16*). When the BNPI mRNA was injected in oocytes, Na^+-dependent uptake of inorganic phosphate was observed, indicating that BNPI is a member of the Na^+-dependent inorganic phosphate transporter family (*16*). Unexpectedly, however, BNPI is specifically expressed and localized in the synaptic vesicles of glutamatergic neurons, suggesting its presynaptic role(s) in glutamatergic neurotransmission (*17*, *18*). It has also been reported that EAT4, a homologue of BNPI, is necessary for glutamatergic neurotransmission in *Caenorhabditis elegans* (*19*). In 2000, two groups independently showed that upon expression in neuroendocrine cells, BNPI is localized with secretory vesicles and facilitates ATP-dependent vesicular L-glutamate uptake, demonstrating that BNPI is VGLUT itself (*4*, *5*). Furthermore, BNPI-expressing neuroendocrine cells can secrete L-glutamate, and the released L-glutamate can excite neighboring cells expressing L-glutamate receptor, indicating that BNPI can be regarded as a glutamatergic phenotype-conferring protein (*5*).

Isoforms of VGLUTs

Not all glutamatergic neurons express BNPI (*4*, *5*, *17*, *18*, *20*): about half of the known glutamatergic neurons are devoid of BNPI, suggesting the presence of another VGLUT moiety in BNPI-deficient neurons. Simultaneously, Aihara et al. cloned a cDNA homologous to that of BNPI as an up-regulated cDNA in the process of differentiation of AR42J cells originating from rat pancreas into neuroendocrine cells, and called it as differentiation-associated Na^+-dependent Pi transporter (DNPI) (*21*). When expressed in various clonal cells, DNPI exhibited VGLUT activity like BNPI, demonstrating that DNPI is another VGLUT (*22*–*27*). Successively, another VGLUT has been identified, which is homologous to BNPI and DNPI, and called as VGLUT3 (*28*–*31*). Thus, VGLUT constitutes a family comprising VGLUT1, 2, and 3 (Fig. 2). Judging from their primary amino acid sequences, VGLUTs belong to the SLC17 type I phosphate transporter family, and seem to be 12 transmembranous proteins with both amino- and carboxyl terminals facing the cytoplasm (*32*) (Fig. 3).

VGLUTs are unique transporters, since they transport L-glutamate electrophoretically when present in synaptic vesicles, while they co-transport Na^+ and inorganic phosphate in the reversed direction when present on the plasma membrane, as described above (*4*, *5*, *21*–*27*) (Fig. 3). Thus, VGLUTs are versatile in nature in their coupling ions, substrate specificity and direction of transport. The structural bases of the versatility, even the amino acid residues responsible for the transport, are totally unknown at present and await further study.

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Fig. 2. **Alignment of the amino acid sequences of VGLUT1, 2, and 3.** Conserved amino acid residues among VGLUTs are boxed.

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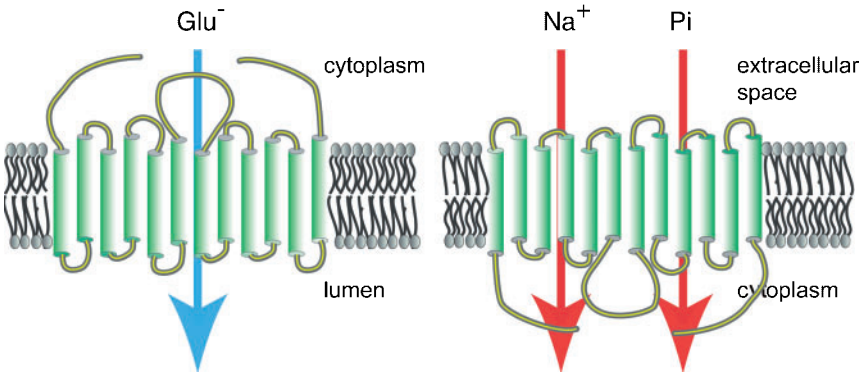

Fig. 3. **Versatile nature of VGLUT1 and VGLUT2.** When present in secretory vesicles, VGLUTs use the membrane potential as a driving force and transport the anionic form of L-glutamate. By contrast, when present on the plasma membrane, VGLUT1 and VGLUT2 co-transport inorganic phosphate and Na^+ in the reverse direction to glutamate transport.

VGLUTs in CNS

VGLUT1 and 2 are expressed exclusively in glutamatergic neurons in CNS, and are reliable markers for glutamatergic excitatory neurons. So far, VGLUT1 and VGLUT2 have not been detected in nonglutamatergic neurons such as GABAergic, serotonergic, cholinergic ones in CNS. VGLUT2 is expressed in C1 adrenergic and nonaminergic presympathetic vasomotor neurons in the medulla. The authors suggest that these neurons may primarily use glutamate as a transmitter, and the C1 adrenergic phenotype may be one of several secondary phenotypes (*33*, *34*).

As expected, VGLUT1 and 2 are complimentarily localized in CNS. VGLUT1-expressing neurons do not possess VGLUT2, and *vice versa*. mRNA for VGLUT1 is mainly expressed in the cerebral cortex, hippocampus, and amygdala, while that for VGLUT2 is predominantly localized in the diencephalic zone (thalamus, hypothalamus, *etc.*) and brain stem region (*22*, *26*, *35*–*39*). In the cerebellum, VGLUT1 is localized in granule cells (parallel fibers), while VGLUT2 is localized in the inferior olivary nucleus (climbing fibers) (*40*, *41*). In the spinal cord, VGLUT2 is predominant (*25*), and the mRNAs for the two transporters are distributed differently (*42*). In rare cases, such as in the medial habenular nucleus in the thalamus, both VGLUTs are expressed (*22*, *26*, *27*, *35*, *37*).

On immunohistochemistry at the light microscopic and electron microscopic levels, VGLUT1, 2 polypeptides were found to be localized in synaptic vesicles (*22*, *26*, *27*, *35*, *36*), and the immunoreactivity reflects the presence of excitatory glutamatergic synaptic terminals of CNS and afferent fibers. Immunoreactivity for VGLUT1 and 2 is also complimentarily distributed in CNS (*22*, *26*, *27*, *35*–*39*). For instance, in the cerebral cortex, VGLUT1 immunoreactivity is distributed throughout the cortex, but VGLUT2 immunoreactivity is localized in layers IV and

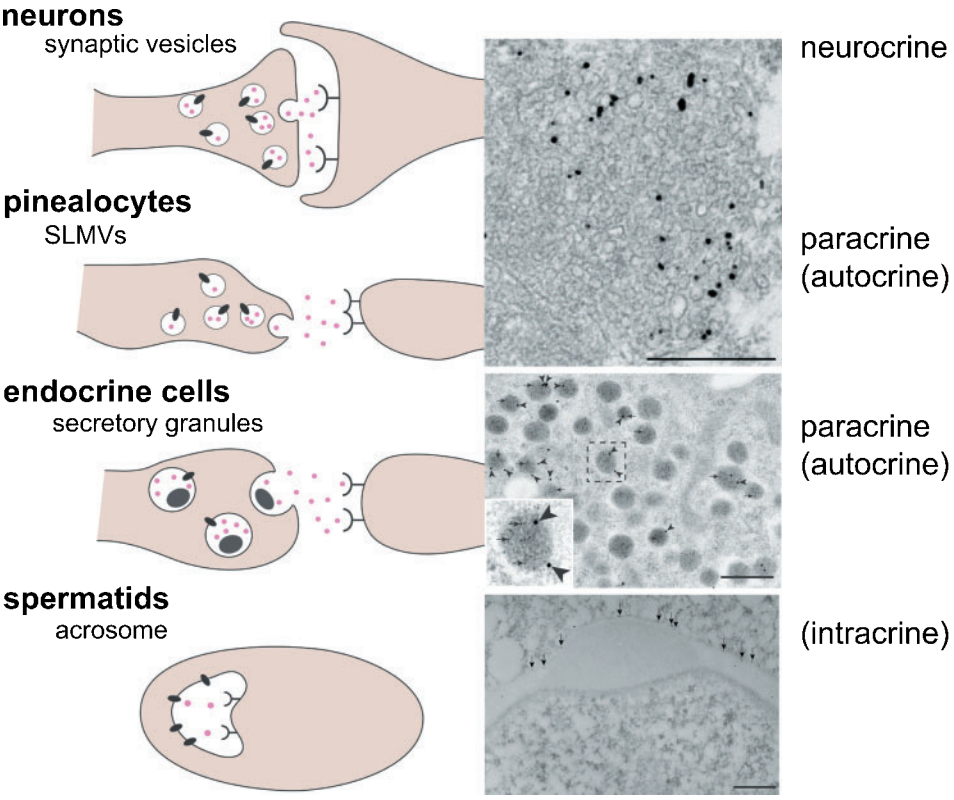

Fig. 4. **The localization of VGLUTs indicates diverse modes of glutamatergic signaling.** For immunoelectronmicroscopy, the silver enhancing method (pineal glands) or LR White embedding method (islet α cells and acrosome) was used (*58*, *60*). Bar = 500 nm.

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VI, suggesting that the axon terminals of cortical or intracortical fibers use VGLUT1 while thalamocortical axon terminals use VGLUT2 as a vesicular glutamate transporter (*22*, *26*, *27*, *35–39*).

This complementary localization of VGLUTs suggests some functional differences between the transporters. It has been reported that the expression of only VGLUT2 increases in the thalamus in schizophrenia (*43*). It would be interesting to determine how VGLUT1 and 2 share physiological and pathological roles in CNS.

On the other hand, VGLUT3 is expressed in the striatum, hippocampus, cerebral cortex, raphe nucleus in the pons, *etc.* (*28*, *29*, *31*). VGLUT3 is expressed in GABAergic neurons, cholinergic neurons and serotonergic neurons in the hippocampus, striatum and raphe nucleus, respectively (*28*, *29*, *31*). Electron-microscopically, VGLUT3 polypeptide is localized in the internal membranous structure near synaptic contact in the dendrites as well as in synaptic vesicles (*29*). VGLUT3 is also expressed in a subset of astrocytes throughout the brain (*29*). This unexpected distribution of VGLUT3 suggests a novel mode of glutamate signaling. It is noteworthy that VGLUT3 functions in peripheral tissues, since expression of the VGLUT3 gene as well as the VGLUT3 polypeptide were detected in kidney, liver and so on (*29*).

VGLUTs in peripheral nervous system (PNS)

VGLUTs are also widely distributed in PNS, such as in the retina (*29*, *44*), trigeminal ganglion (*45*), dorsal root ganglia (DRG) (*42*, *46*), intraganglionic laminar endings (IGLEs) of esophagus (*47*) and enteric neuron (*48*). In the retina, VGLUT1 is expressed in photoreceptor cells and bipolar cells, while VGLUT2 is expressed in ganglion cells (*44*). These cells are well known to use L-glutamate as a transmitter. VGLUT3 is localized in a previously unidentified subset of amacrine cells (*29*). Coincided with the expression of VGLUT2 in ganglion cells of the retina, immunoreactivity of VGLUT2 is dominant in the dorsolateral geniculate nucleus and the superior colliculus, where retinal ganglionic cells terminate. While brainstem auditory and vestibular systems receive VGLUT1 synaptic inputs as major inputs (*27*).

VGLUT1 and 2 are co-expressed with substance P in nociceptive primary afferent fibers terminating in the medullary and spinal dorsal horns, suggesting their role(s) in nociception (*46*). Intraganglionic laminar endings (IGLEs) are putative vagal mechanosensor terminals derived from nodose ganglion. The presence of VGLUT2 in IGLEs suggests a role of L-glutamate as a local signal molecule to myenteric neurons from IGLEs (*47*). In the brain-gut axis, VGLUT2 is expressed in the enteric neurons (submucosal and myenteric plexus), nodose, dorsal root ganglia neurons (*48*). It is suggested that glutamate signaling is used as afferent transfer of information from the mucosa to the enteric plexuses and brain (*48*).

VGLUTs in non-neuronal cells

Various peripheral non-neuronal cells such as bone cells, islet cells, and pineal cells express functional glutamate receptors (*49*). Although the results suggest occurrence of glutamatergic signaling in areas close to these peripheral cells, a critical issue is how L-glutamate appears in the area close to a receptor so as to trigger the glutamatergic response in the target cells. It is possible that L-glutamate comes from the innervated glutamatergic nerve ending through a usual exocytic pathway. It is also possible that neighboring non-neuronal cells secrete L-glutamate. In the latter case, L-glutamate should act as an intercellular messenger between the putative glutamate-secreting non-neuronal cells and receptor-expressing target cells in the peripheral tissues. We designated such a putative glutamatergic signaling pathway as a peripheral glutamatergic system (*1*, *50*). It was found that L-glutamate-secreting non-neuronal cells express VGLUTs, as potential markers for the peripheral glutamatergic system (*1*), which is described in some detail below.

Pineal gland

The first firm evidence of such non-neuronal glutamatergic system was obtained for the pineal gland, an endocrine organ for melatonin, a circadian rhythm hormone. Pinealocytes are parenchymal pineal cells and possess a large number of synaptic-like microvesicles (SLMVs) with diameters of around 50–80 nm (*51*). The SLMVs are structurally and functionally similar to the synaptic vesicles of glutamatergic neurons, since the organelles maintain a luminal pH of around 5.1, which is established by vacuolar H^+-ATPase (*51*), and accumulate L-glutamate at the expense of the membrane potential (positive inside) as a driving force (*50*). The L-glutamate is then secreted through exocytosis (*50*). Recently, it was found that pineal SLMVs contain both VGLUT1 and 2, as revealed by biochemical, immunohistochemical and electronmicroscopical evidence (*52*).

The physiological significance of the glutamate secreted from pinealocytes has been determined to some extent. Pinealocytes express metabotropic glutamate receptor types 3 and 5, and GluR1 AMPA type ionotropic receptors (*50*, *53*). Stimulation of mGluR3 causes inhibition of melatonin synthesis through an inhibitory cAMP cascade: the decreased intracellular cAMP content in turn inhibits transcription of the serotonin *N*-acetyltransferase (AA-NAT) gene, causing decreased AA-NAT activity and decreased melatonin output (*50*). Stimulation of GluR1 receptors may trigger glutamate secretion through an increase in intracellular $[Ca^{2+}]$ (*53*). Thus, glutamate signaling can spread throughout the gland via glutamate-mediated paracrine-like cell to cell interactions. The glutamate signaling can be terminated through plasma membrane glutamate/aspartate transporter, since pinealocytes express GLT-1 type transporter on the plasma membrane (*50*). Together, pineal cells possess the complete set of glutamatergic systems, which is involved in the regulation of melatonin synthesis.

Islets of Langerhans

The second example of peripheral glutamatergic systems is in the islet of Langerhans, a miniature endocrine organ for hormones regulating blood glucose. The islets contain at least four kinds of endocrine cells, *i.e.* glucagon-secreting α cells, insulin-secreting β cells, somatostatin-secreting δ cells, and pancreatic polypeptide-containing F cells. Recent evidence indicates that islet cells express various functional glutamate receptors and Na^+-

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dependent glutamate transport activities, and that stimulation of these receptors affects the secretion of insulin and/or glucagon, suggesting the occurrence of glutamatergic signaling in islets (*1*, *54*, *55*). However, the glutamatergic responses in isolated islet cells, islets, clonal cells, and perfused pancreas upon the addition of an agonist of glutamate receptors are not always consistent and sometimes contradictory (*1*). Thus, the site of glutamate signal appearance in islets should be an important issue for determining the complicated features of the glutamatergic systems in islets. Immunohistochemical, electronmicroscopical, and biochemical evidence indicates that both VGLUT1 and 2 are present in glucagon-containing secretory granules in α cells, suggesting that *L*-glutamate is co-stored and co-secreted with glucagon upon low glucose stimulation (*56*). In fact, L-glutamate and glucagon were secreted when islets were first incubated under high glucose conditions and then transferred to low glucose conditions to trigger glucagon secretion, the molecular ratio being around 2,000/1 (*56*, *57*). Thus, α cells are glutamatergic endocrine cells. VGLUT1 and 2 are also expressed in F cells, and associated with pancreatic polypeptide-containing secretory granules, suggesting that F cells are also glutamatergic, and that pancreatic polypeptides and L-glutamate are co-secreted under low glucose conditions (*58*). By contrast, since neither VGLUIT1, 2 nor 3 is expressed in β and δ cells, these cells are not regarded as glutamatergic cells (*1*).

In islets, glutamatergic signaling may occur from α cells under low glucose conditions. What happens upon the appearance of glutamate signals in islets? Stimulation of AMPA-type ionotropic glutamate receptors on β cells enhanced the exocytosis of GABA-containing SLMVs, but not of insulin granules, causing increased secretion of GABA (*56*). The released GABA in turn may inhibit glucagon secretion through inhibition of exocytosis of glucagon granules by way of GABAA receptors on α cells (*59*). Alternatively, L-glutamate may act as an autocrine-like signaling molecule and inhibit glucagon secretion by inhibiting the exocytosis of glucagon granules through an inhibitory cAMP cascade by way of class III metabotropic glutamate receptors type 4 (mGluR4) on α cells (*60*). Thus, the glutamatergic signaling in islets seems to be involved in the regulation of glucagon secretion. Whether or not the glutamatergic system participates in insulin secretion is unknown at present, further study being awaited. It is noteworthy that the glutamate signal reception on β cells is also dependent on the glucose conditions and occurs only when islet cells are incubated under low glucose conditions. This phenomenon seems to be similar to the sensitization/desensitization state of AMPA-type GluR2 receptors in neurons. Since a neuronal AMPA receptor is recycled depending on the sensitization/desensitization state (*61*), similar recycling might also be dependent on the glucose conditions, which needs further study.

Intestine and stomach

In the gastrointestinal tract, there are more than 20 kinds of endocrine cells. Some of them express VGLUT2 (*58*). Intestinal L cells secrete glucagon-like peptide 1 (GLP-1), a hormone that stimulates insulin secretion from β cells. Immunohistochemical studies have demonstrated that VGLUT2 is present in GLP and polypeptide YY (PYY)-containing secretory granules of L cells. Thus, like islet α cells, L-cells may be glutamatergic, and co-secrete L-glutamate and GLP-1. Glutamate receptor-expressing cells can be observed in close proximity to L cells, suggesting the role(s) of L-glutamate as a paracrine signaling molecule in the ileum (Morimoto, R., Hasyashi, M., and Moriyama, Y., unpublished observation). The small intestine also possesses pancreatic polypeptide-containing endocrine cells. Unlike islet F cells, however, these cells do not express any VGLUTs. The stomach mucosa, especially the antrum and pylorus regions, contains VGLUT2. In contrast with in the small intestine, VGLUT2 coincides with pancreatic polypeptides, but not glucagon, somatostatin, serotonin, or gastrin. Thus, VGLUT2 seems to be associated with pancreatic polypeptide-containing secretory granules, as in the case of islet F cells.

Bone

Osteoblasts and osteoclasts express glutamate receptors and GLAST-type Na^+-dependent glutamate transporter, suggesting a role(s) of L-glutamate in bone metabolism, especially in the maintenance of bone mass under normal or injured conditions (*62*, *63*). Innervation by neurons that exhibit L-glutamate immunoreactivity in their process terminal region has been detected in bone, suggesting that L-glutamate originating from nerve endings stimulates glutamate receptors (*64*). By contrast, Skerry and his colleagues reported that osteoblasts actually secrete L-glutamate, possibly through exocytic processes (*65*). Consistently, osteoblasts express the VGLUT1 gene, which was observed on RT-PCR analysis, although evidence for VGLUTs at the protein level is lacking (*66*). Glutamatergic stimulation has been shown to modulate bone cells, although there are counterarguments, and the signaling pathway is less characterized (*62*). Thus, the glutamatergic system in bone tissue is still at a preliminary stage, and firm evidence for L-glutamate exocytosis and identification of VGLUT-associated organelles is required.

Testes

Mammalian testes consist of many seminiferous tubules, where the germ cells reside at the tubule periphery and give rise to proliferating spermatogonital cells. Only the primitive spermatogonia undergo a series of synchronous cell divisions and differentiation, leading to mature sperm. Immunohistochemical evidence indicates the expression of various glutamate receptors in testes, suggesting the occurrence of glutamatergic systems (*67*, *68*). Very recently, two groups independently detected the expression of VGLUTs in spermatids (*58*, *69*). Redecker and his colleagues reported the expression of VGLUT1 (*69*), while we detected the expression of VGLUT2 but not VGLUT1, on RT-PCR, Northern blotting, Western blotting, and immunohistochemistry with specific antibodies against VGLUT1 and 2 (*58*). At present we do not know the reason for the discrepancy, but we should point out that commercial antibodies sometimes recognize both VGLUT1 and 2. VGLUT2 immunoreactivity is co-localized with acrosin, a marker for acrosomes, indicating the association of VGLUT2 with acrosomal membrane (*58*).

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Immunoelectronmicroscopy revealed that VGLUT2 is predominantly present on the outer acrosomal membrane. Interestingly, GluR5 and KA1, the functional units of kainate receptors, are both expressed and present in the inner acrosomal membrane. Overall, we suppose that the acrosomes are the site of glutamate storage, and that acrosomal L-glutamate in turn stimulates kainate receptors. Such putative signaling may occur in intracellular organelles, thereby, constituting an intracrine signaling (*70*). The expression of VGLUT2 seems to be developmentally regulated, being maximal at an early stage and then decreasing rapidly during maturation, suggesting the importance of the putative glutamatergic signaling in spermatogenesis. The glutamatergic signaling changes to GABAergic signaling during spermatogenesis, since GABAergic systems, *e.g.* GABA receptors and vesicular GABA transporter, appear after the expression of VGLUTs has decreased (Morimoto, R., Hayashi, M., Yamamoto, A., and Moriyama, Y., manuscript in preparation). The role(s) of L-glutamate in spermatogenesis is totally unknown at present, further studies being awaited.

Diverse modes of glutamatergic signaling

The detection of VGLUTs in non-neuronal peripheral tissues conclusively demonstrated the presence of peripheral glutamatergic systems, which revealed a novel feature of glutamatergic signaling. Peripheral glutamatergic systems may use L-glutamate as an intercellular messenger to communicate with neighboring cells. Thus, the peripheral glutamatergic systems indicate that glutamatergic intercellular transmitters are not restricted to neuronal chemical transmission but one of the general and ubiquitous systems for intercellular chemical transmission. Furthermore, as summarized in Fig. 4, glutamatergic systems in CNS, PNS, and peripheral non-neuronal cells reveal that the mode of glutamate signal transmission is diverse in nature. For instance, the storage organelles for L-glutamate, stimulus/exocytosis coupling, kinetics of the appearance of glutamate signals, and the distance from the target cells are different. Different sizes of storage organelles may cause different concentrations of internal L-glutamate. Different kinetics of exocytosis may cause different kinetics of glutamate signal transmission. These results imply that the mode of glutamatergic signaling is diverse in nature. More refined studies on the glutamatergic signaling in PNS and peripheral tissues will be helpful for a full understanding of the glutamatergic signaling.

Perspectives and concluding remarks

The identification of the VGLUT moiety will tremendously facilitate study on their structure and function, although such studies are at a preliminary stage at present. Many fascinating issues remain to be solved, *i.e.* structure of VGLUTs, the molecular basis for the transport phenotypes and targeting to the synaptic vesicles, and so on. In particular, targeting of the VGLUT moiety to synaptic vesicles must be one of the essential steps for appearance of glutamatergic phenotype in neurons. Such information should be inherent in the VGLUT moiety, which is one of the urgent issues.

At present, there is no heterogeneity of VGLUTs as to glutamate transport activity. The complementary expression of VGLUT1 and 2 in CNS, however, suggests the different significance of each VGLUT as to glutamatergic signaling. At least, the complementary expression of VGLUTs is useful for classifying glutamatergic neurons in more detail, which will will reveal the features of the glutamatergic neurons in more detail. The VGLUTs in non-neuronal cells unexpectedly reveal a wider distribution of glutamatergic signaling. As described above, VGLUT3 is also expressed in peripheral tissues such as kidney and liver. However, the precise localization of VGLUT3 at the cellular or subcellular level is unknown at present, further study being awaited.

In conclusion, the identification of the VGLUT moiety will no doubt lead to a new era as to the glutamatergic signaling. Glutamatergic signaling occurs in not only in CNS but also PNS and non-neuronal peripheral tissues. Further studies on VGLUTs will provide insights into the unity and diversity of glutamatergic signaling.

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