Novel Members of the Vesl/Homer Family of PDZ Proteins That Bind Metabotropic Glutamate Receptors*

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Vesl-1S (186 amino acids, also called Homer) is a protein containing EVH1- and PDZ-like domains whose expression in the hippocampus is regulated during long term potentiation (LTP), one form of synaptic plasticity thought to underlie memory formation (Kato, A., Ozawa, F., Saitoh, Y., Hirai, K., and Inokuchi, K. (1997) FEBS Lett. 412, 183–189; Bramke, P. R., Lanahan, A. A., O’Brien, R., Roche, K., Barnes, C. A., Huganir, R. L., and Worley, P. F. (1997) Nature 386, 284–288). Here we report additional members of the Vesl/Homer family of proteins, Vesl-1L and Vesl-2. Vesl-1L (366 amino acids), a splicing variant of Vesl-1S, shares N-terminal 175 amino acids with Vesl-1S and contains additional amino acids at the C terminus. Vesl-2 (354 amino acids) was highly related to Vesl-1L in that both contain EVH1- and PDZ-like domains at the N terminus (86% conservation) and an MCC (mutated in colorectal cancer)-like domain and a leucine zipper at the C terminus. In contrast to vesl-1S, we observed no changes in the levels of vesl-1L and vesl-2 mRNAs during dentate gyrus LTP. All these proteins interacted with metabotropic glutamate receptors (mGluR1 and mGluR5) as well as several hippocampal proteins in vitro. Vesl-1L and Vesl-2, but not Vesl-1S, interacted with each other through the C-terminal portion that was absent in Vesl-1S. Vesl-1L and Vesl-2 may mediate clustering of mGluRs at synaptic junctions. We propose that Vesl-1S may be involved in the structural changes that occur at metabotropic glutamergic synapses during the maintenance phase of LTP by modulating the redistribution of synaptic components.

Hippocampal long term potentiation (LTP),1 which is one form of synaptic plasticity thought to underlie cellular mechanisms of learning and memory (1), has two distinct phases. The early phase persists for several hours and does not require macromolecule synthesis, whereas the late phase lasts for weeks in vivo and depends on de novo protein and RNA synthesis for its maintenance (2–7). This indicates that a particular set of genes, up-regulated following LTP induction, play an important role in the maintenance of late LTP.

vesl (VASP/Ena)-related gene up-regulated during seizure and LTP/homer was isolated as a synaptic plasticity-regulated gene from rat hippocampus (8, 9). Hereafter, we denote the vesl cDNA that codes for a protein of 186 amino acids as vesl-1S. The expression of the vesl-1S transcript is induced in the granule cell layer of the dentate gyrus of both freely moving unanesthetized and urethane-anesthetized rats following a delivery of high frequency stimuli (HFS) to the perforant pathway, which elicits a persistent LTP lasting either several weeks or ~20 h, respectively. The induction is N-methyl-D-aspartate (NMDA) receptor-dependent. The Vesl-1S protein has significant homology to the EVH1 domain of VASP/Ena family of proteins, which is implicated in the control of actin filament dynamics (10). The EVH1 domain of Mena, a mammalian homolog of Drosophila Ena, is thought to mediate targeting of Mena to focal contacts by interacting with zyxin, and probably with vinculin, which is localized to the focal contacts.

The Vesl-1S protein also contains a PDZ-like domain within the EVH1 homologous region. The PDZ domain constitutes an interface for certain kinds of protein-protein interactions, specifically interactions with integral membrane proteins such as receptors (for reviews, see Refs. 11–13). For example, PDZ domains of PSD95/SAP90 bind the R2 subunit of the NMDA receptor and a subunit of the Shaker K+ channel (14, 15). One of the well known functions of PDZ proteins is their ability to cluster associated integral membrane proteins at synapses (14, 16). The PDZ-like domain of Vesl-1S/Homer interacts with mGluR1 and mGluR5 (9). Vesl-1S/Homer is the first PDZ-domain protein that is found to be up-regulated by extracellular stimulation such as LTP. Thus, Vesl-1S/Homer may function as a modulator for mGluR signaling. Here we report additional members of the Vesl-1S/Homer family of mGluR-binding proteins.

EXPERIMENTAL PROCEDURES

Cloning and Sequencing of vesl-1L and vesl-2 cDNAs—Reverse-transcription-polymerase chain reaction (RT-PCR) was carried out as described (17). Plaque hybridization was performed with a ZAP-PII cDNA library constructed from rat hippocampal mRNA (Stratagene) (18). The DNA probes for plaque hybridization were labeled with [α-32P]dCTP using a random-prime labeling kit (Stratagene). The nucleotide sequence was determined on both strands by the dideoxy chain-termination method using automatic DNA sequencers, ALFII (Amersham Pharmacia Biotech) and PRISM™ 373 (Applied Biosystems).

Glutathione S-transferase (GST) and Maltose-binding Protein (MBP) Fusion Constructs—DNA fragments covering the entire coding region of vesl-1S, vesl-1L, and vesl-2 were amplified with PCR and introduced into the EcoRI and SalI sites of pGEX 5X-1 (Amersham Pharmacia

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Mammalian Expression Constructs—FLAG (DYKDDDDK)- or Myc (EQKLISEEDL)-tagged Vesl constructs were generated by PCR using specific primers and subcloned into the mammalian expression vector pcDNA3 (Invitrogen), which contains cytomegalovirus promoter. FLAG- and Myc-epitope tags were inserted at the initiation codon and codon for the second amino acid to construct FLAG-Vesl-1S and Myc-Vesl-2, respectively. To generate Vesl-1S-FLAG and Vesl-2-Myc, FLAG and Myc tags were added at the C-terminus, respectively.

In Vivo Interaction of Vesl-1L and Vesl-2—Transfections to COS-7 cells were performed with 50 μg of each plasmid using the electroporation method (Gene Pulser™, Bio-Rad) according to manufacturer’s instructions. After forty-eight hours after the transfection, cells were lysed in lysis buffer (1% Triton X-100, 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM Pefabloc SC) at 4 °C for 1 h. The lysates were centrifuged for 15 min at 10,000 g to remove cell debris. One μg of monoclonal antibody (anti-FLAG M2 antibody, Eastman Kodak Co.; anti-Myc antibody IgG2b, kindly supplied by Dr. Masami Takahashi; or control IgG, IgG1 from mouse MOPC 21, Organon Teknika Co.) was added to each cell lysate (400 μg of proteins), and the lysates were incubated for 1 h at 4 °C. Immunoprecipitation was performed by adding 30 μl of 30% protein G-agarose (Falcon), followed by incubation for 1 h at 4 °C and a brief centrifugation. The precipitates were washed three times with lysis buffer and subjected to SDS-PAGE. Immunoblot was performed as described above except anti-FLAG or anti-Myc monoclonal antibody.

Dentate Gyrus LTP—All the LTP experiments were performed on freely moving unanesthetized animals. Adult male Wistar rats (16–20 weeks old, >400 g) were implanted with electrodes made of tungsten wire under pentobarbital anesthesia (50 mg/kg body weight, intraperitoneally) as described previously (8). Coordinates were as follows: bipolar stimulating electrode, 8.0 mm posterior, 4.5 mm lateral, and 5.0 mm inferior to bregma; recording electrode, 4.0 mm posterior, 2.5 mm lateral, and 3.8 mm inferior to bregma ipsilaterally. After the surgery, each animal was allowed to recover in an individual home cage for at least 3 weeks.

All the stimuli were constant current, biphasic rectangular pulses (200-μs width). During recording and tetanic stimulation, the animals were placed to a slipring connector so that the animals were able to move freely. One day before the LTP experiment, an input-output curve was determined by delivering biphasic pulses starting from an intensity of 100 μA with steps of 100 μA. For each intensity, 5 pulses at a frequency of 0.1 Hz were applied, and the evoked response was then averaged. Following a 5-min interval, the stimulation intensity was increased by 100 μA. A stimulation intensity was selected that evoked an averaged field excitatory postsynaptic potential (fEPSP) that was approximately 50% of its maximal amplitude. This intensity was used for test and tetanic stimuli. LTP was induced by HFS following a 20-min base-line recording. HFS (total of 2400 pulses) consisted of 4 bursts delivered at a 15-min interburst interval. Each burst consisted of 20 trains delivered at 5-s intertrain intervals (each train, 400 Hz, 30 pulses). The evoked field potentials were amplified, digitized at 50 kHz, and stored sequentially on computer disks (NEC PC9801 DX) for off-line analysis. The maximal fEPSP was defined as the field excitatory postsynaptic potential and population spike amplitude were monitored as an index of LTP.

In Situ Hybridization—Animals were deeply anesthetized either with pentobarbital (50 mg/kg, intraperitoneally) for LTP animals or with diethyl ether for pups. Brains of anesthetized LTP animals were prefixed by cardiac perfusion with 4% paraformaldehyde. Anesthetized pups were decapitated, and brains were dissected. Dissected brains from LTP animals and pups were fixed in 4% paraformaldehyde for 15 h at 4 °C and then embedded in O.C.T. compound (Miles). In situ hybridization was carried out essentially as described (17) using digoxigenin-labeled cRNA probes, which were designed to hybridize specifically to vesl-1S, vesl-1L, or vesl-2.

RESULTS

Structure of Vesl-1L and Vesl-2 Proteins—Western blot analysis with anti-Vesl-1S antisem showed that hippocampal lysate contained a protein of expected size (28 kDa) of Vesl-1S (Fig. 1). The intensity of the band increased following kainate treatment, indicating that the band size corresponds to Vesl-1S protein. The Western blot showed an additional signal of 45 kDa, the level of which, in the hippocampus, was not increased by kainate treatment. This strongly suggests the presence of either the splicing variant or homologous protein of Vesl-1S in the hippocampus. We examined these possibilities by screening a hippocampal cDNA library.
The hippocampal cDNA library was screened with probe A that encompasses the entire coding region of vesl-1S (Fig. 2A). Forty cDNA clones that hybridized with probe A were obtained by screening approximately 10^6 plaques. A DNA sequence analysis of 9 of these revealed that 2 codons for the Vesl-1S protein, whereas the remaining 7 corresponded to an alternatively spliced cDNA. This cDNA, termed vesl-1L, encoded a protein of 42 kDa (366 amino acids) that shares the N-terminal 175 amino acids with the Vesl-1S protein and contains an additional 191 residues at the C terminus (Fig. 2B).

As an alternative method of isolating the vesl-1S homolog, we employed the RT-PCR method. From a DNA data base search, we observed that several expressed sequence tags (ESTs) isolated from mouse and human encoded a protein homologous to, but distinct from, Vesl-1S. This was also observed by Brakeman et al. (9). According to the nucleotide sequence of the mouse EST, a pair of PCR primers was designed to amplify a rat counterpart of the mouse EST (5'-AGGAACAGCTATCGGATCATCAGTGT-3' and 5'-CAGCTCCA-TCTCCACACTTCCAC-3'). An RT-PCR fragment amplified from the total RNA, prepared from the adult rat hippocampus with this primer pair, was used as a probe to screen a rat cDNA library. By these means, a novel cDNA, vesl-2, was isolated, which coded for an additional member of the Vesl family (354 amino acids) (Fig. 2B). We also found an alternative spliced form of Vesl-2 (Vesl-2α11) in which 11 amino acid residues (129–139) were deleted.

In agreement with the apparent molecular mass from the Western blot analysis (45 kDa), vesl-1L and vesl-2 cDNAs encode protein products with an expected molecular mass of 42 kDa. In fact, the heterologously expressed vesl-1L and Vesl-2 proteins in cDNA-transfected COS-7 cells migrated at the same size as the band in the brain (45 kDa) (see Fig. 6). Whereas the N-terminal half of Vesl-1L (amino acids 1–175) shares exactly the same amino acid sequence with Vesl-1S, the C-terminal half contains an additional polypeptide, indicating that Vesl-1L is a splicing variant of Vesl-1S. Vesl-2 has a similar structure in that it has a highly Vesl-1S-homologous region at the N terminus (86% identical in 120 amino acid residues) and an extra polypeptide at the C terminus. The EVH1-homologous region is conserved both in Vesl-1L and Vesl-2 proteins. Importantly, Vesl-1S, Vesl-1L, and Vesl-2 all contain the RXXXXYGLGF motif that is conserved in all the PDZ proteins reported and that, in the case of PSD95/SAP90, constitutes a binding pocket for the NMDA receptor (19) (Fig. 2B). The homology in the amino acid sequences between Vesl-1L and Vesl-2 decreases at the C-terminal half but is still significant (34% identical in 246 amino acids).

Specifically, the C-terminal end containing the leucine zipper was highly conserved, suggesting that Vesl-1L and Vesl-2 form a dimeric structure. Both proteins have putative phosphorylation sites for protein kinase A and protein kinase C. A unique characteristic of Vesl-1L and Vesl-2 as a PDZ protein is that they lack both the Src homology 3 region and a domain homologous to the low molecular weight guanylate kinases. A search in the GenBank™ DNA data base revealed an existence of a homologous protein from Drosophila (Fig. 2B). The structure of Vesl family has been highly conserved throughout evolution. The search further showed that both Vesl-1L (amino acid residues 195–245) and Vesl-2 (183–233) have a homologous region to the MCC protein, a putative colorectal tumor suppressor (20) (Fig. 2C). The corresponding region of MCC has similarity with the G protein-activating region of the m3 muscarinic acetylcholine receptor and is implicated in the G protein-mediated response (20).

Vesl-1L and Vesl-2 Interacted with mGluR1 and mGluR5—Vesl-1S/Homer interacts with mGlur1 and mGlur5 in vitro (9). We reasoned that both the Vesl-1L and Vesl-2 proteins also interact with mGlur5, because both proteins have a PDZ-like motif and contain the Vesl-1S-conserved region that binds to the receptors. A solution binding assay demonstrated that all these proteins interacted with mGlur1 and mGlur5 (Fig. 3). GST-Vesl-1S, GST-Vesl-1L, and GST-Vesl-2 fusion proteins expressed in bacteria bound both mGlur1 and mGlur5 in hippocampal extract.

Vesl-1L and Vesl-2, but Not Vesl-1S, Interacted with Each Other—We employed Far-Western blot analysis to elucidate the functional role of the C-terminal region of Vesl-1L and Vesl-2. Proteins from the adult hippocampus were separated by SDS-PAGE, transferred onto a membrane filter, and overlaid with GST fusion proteins. Binding of the fusion proteins was detected with anti-GST antibody (Fig. 4). GST-Vesl-1L and GST-Vesl-2, but not GST-Vesl-1S, fusion proteins bound to the 45-kDa protein(s) in the brain extract, suggesting that the C-terminal portion of Vesl-1L and Vesl-2 mediates the interaction with the 45-kDa protein(s). In fact, the C-terminal portion of Vesl-1L and Vesl-2 fused to GST (GST-Vesl-1L(C) and GST-Vesl-2(C), respectively) bound to the 45-kDa protein(s).

Because the migration of the 45-kDa protein(s) on SDS-PAGE was the same as that of Vesl-1L and Vesl-2 proteins (not shown), we examined whether the 45-kDa protein(s) were actually Vesl-1L or Vesl-2. The bacterially expressed Vesl family, fused to MBP, was subjected to the Far-Western blot analyses with purified GST fusions (Fig. 5). Signals were visualized with anti-GST antibody. GST fusions of Vesl-1L, Vesl-2, and Vesl-2α11 bound to the MBP fusions of Vesl-1L, Vesl-2, and Vesl-2α11 but not to the MBP-Vesl-1S fusion. Vesl-1L prefers to bind heterophilically compared with Vesl-2, which bound both homo- and heterophilically. GST and GST-Vesl-1S failed to bind any of the MBP fusions. The C-terminal portion of either Vesl-1L or Vesl-2 was sufficient for these interactions. Thus, Vesl-1L and Vesl-2 interact with each other in a homophilic and heterophilic manner in vitro.
absent in the precipitate (Fig. 6A). Thus, the coprecipitation of FLAG-Vesl-1L with Myc-tagged Vesl-2 was specific. Similar results were obtained when lysates of COS-7 cells cotransfected with expression plasmids were immunoprecipitated by anti-FLAG antibody (Fig. 6B), where Myc-tagged Vesl-2 coimmunoprecipitated with FLAG-Vesl-1L. When control IgG was used for immunoprecipitation, neither FLAG-Vesl-1L nor Myc-tagged Vesl-2 coimmunoprecipitated. All the immunoprecipitation target proteins were efficiently expressed in COS-7 cells after the transfection (data not shown). Taken together, these results support the notion that Vesl-1L and Vesl-2 exist as a complex in vivo.

In addition to Vesl-1L and Vesl-2 per se, GST fusions of Vesl-1S, Vesl-1L, and Vesl-2 bound to several other proteins (Fig. 4). These interactions were mediated by the N-terminal region of Vesl-1L and Vesl-2, because both GST-Vesl-1L(C) and GST-Vesl-2(C) failed to bind to these proteins. The proteins of 150 kDa might be mGluR1 and mGluR5. The binding profiles for all the GST fusion proteins did not change when we used with expression plasmids were immunoprecipitated by anti-FLAG antibody (Fig. 6B), where Myc-tagged Vesl-2 coimmunoprecipitated with FLAG-Vesl-1L. When control IgG was used for immunoprecipitation, neither FLAG-Vesl-1L nor Myc-tagged Vesl-2 coimmunoprecipitated. All the immunoprecipitation target proteins were efficiently expressed in COS-7 cells after the transfection (data not shown). Taken together, these results support the notion that Vesl-1L and Vesl-2 exist as a complex in vivo.

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the hippocampal lysate prepared from adult rats that had undergone kainate-induced seizure (3 h) (data not shown).

Expression of vesl mRNAs during Dentate Gyrus LTP in Freely Moving Animals—We examined whether the expression of vesl-1L and vesl-2 mRNAs is regulated by persistent dentate gyrus LTP using the specific cRNA probes, probe I, probe II, and probe III (Fig. 2A). A filter hybridization experiment revealed that each probe did not cross-hybridize with any other cDNAs (not shown). In unanesthetized, freely moving animals, repeated HFS (a total of 2400 pulses) given to the perforant pathway elicited a long-lasting (∼2 weeks) LTP, recorded from the dentate gyrus. The vesl-1S mRNA was markedly induced in the granule cell layer of the dentate gyrus ipsilateral to the stimulating electrode 30 min after the delivery of the last tetanic stimulation (Fig. 7A). In contrast, we did not detect any changes in the mRNA levels of vesl-1L and vesl-2 following the HFS. Therefore, vesl-1S mRNA was up-regulated by persistent LTP, whereas vesl-1L and vesl-2 transcripts were constitutively expressed in the adult hippocampus. To compare the amounts of vesl-1S and vesl-1L transcripts during LTP, we next employed a cRNA probe X that consisted of a part of the vesl-1S-coding region and consequently hybridized to both of the mRNAs with equal efficiency (Fig. 2A). Even though probe X could detect both forms, in situ hybridization with probe X showed a marked increase in the signal intensity in the dentate gyrus 30 min after the last tetanic stimulation (data not shown). Therefore, following LTP induction, the expression level of vesl-1S mRNA was significantly higher than that of constitutively expressed vesl-1L mRNA.

Expression of vesl mRNAs during Hippocampal Development—We have previously observed that vesl mRNA is expressed at a high level during hippocampal development between postnatal days 7 and 14 (P7-P14) (8). The probe used for in situ hybridization in reference (8) contained the entire coding region of the vesl-1S (probe A, see Fig. 2A) and thus hybridized with both vesl-1S and vesl-1L. We re-examined the developmental regulation of each mRNA expression using specific cRNA probes (Fig. 7B). The level of vesl-1S mRNA was low throughout the development of the hippocampus, whereas vesl-1L mRNA was expressed at P8. The expression profile of vesl-2 mRNA was similar to that of vesl-1L, relatively high at

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P8 and low at P15 and P22. Taken together, the expression of vesl-1L and vesl-2 mRNAs was regulated during hippocampal development but not during LTP of adult hippocampus. In marked contrast, vesl-1S expression was up-regulated following LTP induction, but not during development.

**DISCUSSION**

The Vesl proteins constitute a novel family of PDZ proteins that bind to mGluR1 and mGluR5, G protein-coupled glutamate receptors that mediate phosphatidylinositol turnover. This family is unique in that it contains an inducible form, the Vesl-1S protein, that is up-regulated during seizure and LTP in the adult hippocampus. This is the first PDZ-domain protein whose expression is regulated by certain kinds of extracellular stimuli. Fig. 8 summarizes the structure of the Vesl family of proteins. Vesl-1S is a splicing variant of Vesl-1L, which lacks the C-terminal portion present in Vesl-1L. The N-terminal portions of Vesl-1S and Vesl-1L (175 amino acids) are derived from the same exon. Vesl-2 is closely related to Vesl-1L. Specifically, the N-terminal portion, which contains the RXXXXXGLGF motif of the PDZ domain and the EVH1-homologous region, is highly conserved between Vesl-1L and Vesl-2. As expected given their structural similarity, all these proteins were able to bind to mGluR1 and mGluR5, probably through the RXXXXXGLGF motif. In contrast, the C terminus was less conserved between Vesl-1L and Vesl-2. Both proteins have a leucine-zipper at the C-terminal end. In fact, they generated a multimeric structure both in a homophilic and heterophilic manner. This interaction was mediated by the C-terminal.

mGluR5 normally forms a disulfide-linked dimer on the membrane that is mediated by cysteine residues located at the N terminus in the extracellular domain (21). Other mGluRs, including mGluR1, also exist as dimers (21). The multimeric structures of both Vesl-1L/Vesl-2 and mGluRs permit a formation of multivalent aggregation. We hypothesize that Vesl-1L and Vesl-2 mediate the formation of mGluR1 and mGluR5 clusters in the synaptic junction. In this model, Vesl-1L/Vesl-2 and mGluRs form multivalent cross-linking, and this in turn generates clustering of the receptors on the plasma membrane. Thus, homo- or heteromultimerization of Vesl-1L and Vesl-2 could contribute to the aggregation of their partner.

In addition to mGluRs and Vesl-1L/Vesl-2, an overlay experiment revealed that the N-terminal portions of the Vesl pro-
teins interacted with several other hippocampal proteins. The N-terminal region of the Vesl family, which contains EVH1-like and PDZ domains, appears to serve as an interface that mediates protein-protein interactions. The EVH1 domain of Mena interacts directly with zyxin, a component of the actin cytoskeleton (10). PDZ domain 3 of PSD95/SAP90 binds to the neural cell surface proteins, neuroglinins (22). These interactions serve as a link between the NMDA receptor and the synaptic cell adhesion molecule. Therefore, it is quite likely that proteins binding to the N terminus of the Vesl family involve cell adhesion molecule(s) and cytoskeletal components. The Vesl family may provide a molecular framework for the assembly of synaptic proteins that are involved in mGluR signaling.

It is unclear how the level of vesl-1S transcript is regulated following LTP induction. Although vesl-1S and vesl-1L share the same exon, which corresponds to the N-terminal end of the protein, the expression of vesl-1L mRNA in the dentate gyrus was not modulated during LTP. One possibility is that the transcription of each mRNA is under the control of the same cis-acting regulatory element/transcriptional promoter, which, during LTP, promotes an elevated transcription of the vesl-1 gene, whereas the regulation of alternative splicing results in the production of different mRNAs. According to this possibility, the level of the LTP, mRNA precursor of the vesl-1 gene will be spliced out to generate mainly vesl-1L mRNA. On the other hand, the induced mRNA precursor will be alternatively spliced to preferentially generate vesl-1S mRNA during long lasting LTP. Another possible way that these mRNAs are produced is that two distinct promoters, one inducible and the other constitutive, may be responsible for the transcription of vesl-1S and vesl-1L, respectively. In this case, the first exons for each mRNA would be different and this might cause a selection of distinct 3′-exons during splicing. These possibilities remain to be examined.

In the developing hippocampus, the expression of vesl-1L and vesl-2 mRNA was high at P8, then decreased along with the progression of hippocampal development. In contrast, the level of vesl-1S transcript was constitutively low and not regulated during hippocampal development. Therefore, during ontogenesis of the nervous system, Vesl-1L and Vesl-2, but not Vesl-1S, may play a role in the formation of metabotropic glutamatergic synapses.

A characteristic feature of Vesl-1S is its inducibility during hippocampal LTP of the adult brain. How does the inducible Vesl-1S protein function during long lasting neuronal plasticity? As reported previously, Vesl-1S contains a PEST sequence that has been thought to mediate rapid protein degradation (8). Although the level of the vesl-1S transcript was significantly higher than the vesl-1L and vesl-2 mRNA levels during LTP and seizure, the protein levels in the hippocampus did not reflect the mRNA levels, i.e. the level of Vesl-1S protein was far less than Vesl-1L and/or Vesl-2 following kainate-induced seizure. This may be due to either the instability of the Vesl-1S protein or an inefficient translation initiation of the vesl-1S transcript. We have examined the latter possibility by introducing the Kozak consensus sequence around the initiation codon of vesl-1S and vesl-1L cDNAs.3 When transfected into Chinese hamster ovary and COS-7 cells the Kozak-vesl-1S plasmid produced a low level of Vesl-1S protein in these cells, whereas the Kozak-vesl-1L plasmid generated a large amount of Vesl-1L protein. This strongly suggests a rapid turnover of the Vesl-1S protein, because efficiency of the translation initiation would be the same for these constructs.

Two characteristics of the Vesl-1S protein, inducibility during LTP and rapid degradation, and the presence of the homologous proteins Vesl-1L and Vesl-2, expressed constitutively, suggest a functional role for Vesl-1S as a dominant-negative regulator of protein-protein interactions in the macromolecular complex, mediated by Vesl-1L and Vesl-2 at the synaptic junctions. Disruption of the interactions caused by increased levels of Vesl-1S could lead to a redistribution of synaptic components, specifically mGluR1 and mGluR5. Redistribution, in turn, could contribute to synapse restructuring associated with the maintenance phase of hippocampal LTP (23). This structural alteration may occur at both postsynaptic and presynaptic sites, because an electron microscopic study of the hippocampus demonstrated the presence of mGluR5 immunoreactivity in dendritic spines and shafts, as well as in presynaptic axon terminals (24). Further isolation and characterization of proteins that interact with the Vesl/Homer family of proteins will provide more insight into the functional role of this family in synaptic plasticity.

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