vesl, a gene encoding VASP/Ena family related protein, is upregulated during seizure, long-term potentiation and synaptogenesis

Akihiko Kato, Fumiko Ozawa, Yoshito Saitoh, Keiko Hirai, Kaoru Inokuchi*

Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan

Received 14 May 1997; revised version received 9 June 1997

Abstract We have isolated a novel cDNA, *vesl*, that was induced during convulsive seizure in the rat hippocampus. The *vesl* gene encodes a protein of 186 amino acids that has significant homology to the EVH1 domain of the VASP/Ena family of proteins implicated in the control of microfilament dynamics. The expression of *vesl* mRNA was induced in the granule cell layer during persistent long-term potentiation (LTP) of the dentate gyrus in an NMDA receptor-dependent manner. Furthermore, *vesl* mRNA was expressed at a high level during hippocampal synaptogenesis. We suggest that the Vesl protein may be involved in the structural changes that occur at synapses during long-lasting neuronal plasticity and development.

Key words: Long-term potentiation; Seizure; Hippocampus; Gene regulation; PCR differential display

1. Introduction

Behavioral and pharmacological studies have revealed that memory has at least two distinct components, short-term and long-term memory. Long-term memory differs from short-term memory in that it requires gene expression and protein synthesis at a critical period during and/or shortly after the learning experience (for review, [3]). Various protein synthesis inhibitors block the prolonged storage of memory without affecting short-term memory, in both vertebrates and invertebrates. This suggests that gene products synthesized during learning switch the stored information from a short-lasting labile form to a long-lasting stable form [4].

Activity-dependent changes in the strength of synaptic transmission have been considered to provide a plausible cellular basis for learning and memory. Long-term potentiation (LTP) is a typical form of synaptic plasticity, in which electrical stimulation causes a long-lasting increase in synaptic efficacy [5]. Importantly, LTP in the hippocampus appears to have two distinct phases. The early phase, which persists ~3 h, is independent of macromolecule synthesis, whereas the late phase, which lasts for several hours in vitro and even for weeks in vivo, is prevented by pharmacological inhibition of protein and RNA synthesis, without any effects on the early phase [6–10]. These results strongly suggest that a particular set of genes, whose expression is induced after neural activity, play crucial roles in the prolonged maintenance of plastic changes in synaptic efficacy. Recent studies have identified a number of genes that are induced following a high-frequency stimulus (HFS) that elicits the late phase of LTP [11–20]. A major class of genes identified so far encodes transcription factors such as NGFI-A (also called Zif268, Krox24 and Egr1), c-Fos, Krox20 (Egr2), and Egr3 [11,12,16,20], supporting the hypothesis that altered gene expression is a prerequisite for the prolonged maintenance of LTP. In addition to these regulatory proteins, the late phase of LTP is accompanied by the induction of effector proteins such as activin βA and Arc [18,19,21]. One of the most important functions of effector proteins is thought to be their contribution to the reorganization of the neural circuit, which they achieve by either strengthening the pre-existing synaptic connections or, perhaps, by promoting the formation of new synaptic connections.

Here we report the identification of a novel plasticity-regulated gene, *vesl* (*V*ASP/*E*na-related gene upregulated during *s*eizure and *L*TP). Structural analysis of the cDNA revealed that the N-terminal half of the predicted amino acid sequence had similarity to the EVH1 domain of the VASP/Ena family of proteins. This class of protein is enriched in focal adhesion and has been implicated in actin dynamics [22,23]. Prolonged maintenance of neuronal plasticity is considered to rely on, at least partly, the formation of new synaptic connections, which requires neurite outgrowth. Neurite outgrowth at growth cones is carried out through actin polymerization at filopodia. We suggest that Vesl may contribute to the perpetuation of the plastic change in synaptic transmission by regulating actin polymerization.

2. Materials and methods

2.1. *mRNA differential display screening of neural activity-dependent genes*

The screening was performed essentially as described previously [19,20].

2.2. *Surgical procedure and electrophysiology*

Adult male Wistar rats (12–18 weeks old, 400–450 g) were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) and placed on a stereotaxic apparatus. The skull was exposed and electrodes were im-

*Corresponding author. Fax: (81) (427) 24-6314.
E-mail: kaoru@libra.ls.m-kagaku.co.jp

Abbreviations: LTP, long-term potentiation; PTZ, pentylenetetrazol; bp, base pair(s); CHX, cycloheximide; kb, kilobase(s); ORF, open reading frame; NMDA, *N*-methyl-D-aspartate; HFS, high-frequency stimulation; LFS, low-frequency stimulation; fEPSP, field excitatory postsynaptic potential; i.p., intraperitoneal; G6PDH, glucose 6-phosphate dehydrogenase

Some of the results described in this work have been published in abstract form, in which *vesl* was called Ti3 [1]. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with the following accession number: AB003726. During preparation of the manuscript, we have noted that Vesl (also called Homer) selectively interacts with metabotropic glutamate receptors [2].

PII S0014-5793(97)00775-8

planted through burr holes in the skull. Stimulating and recording electrodes were made of epoxylite-coated tungsten wire (100 μm diameter). The bipolar stimulating electrode was positioned unilaterally to the perforant pathway at stereotaxic coordinates of 8.0 mm posterior, 4.5 mm lateral, and 4.5 mm inferior to bregma. The bipolar electrode consisted of two parallel strands of the same wire. A monopolar recording electrode was inserted ipsilaterally into the hilus of the dentate gyrus, 4.0 mm posterior, 2.5 mm lateral, and 3.8 mm inferior to bregma. The depth of the two electrodes was adjusted by monitoring the field potential so that the slope of the field excitatory postsynaptic potential (fEPSP) was maximal. The electrodes were connected to miniature sockets and they were fixed to the skull by dental cement (Shofu). Stainless anchor bolts screwed into the skull served as reference electrode and earth. At the end of surgery, the sutured wound margin was sterilized and antibiotic (Teramycin, 20 mg/kg, Pfizer) was injected intramuscularly. Each animal was cared for, and allowed to recover in individual home cage. Food and water were available ad libitum. The colony room had a 12/12 h light/dark cycle and was maintained at 18 ± 2°C.

LTP experiments were carried out under urethane anesthesia. Following a 2- to 3-week recovery period, 25 animals were anesthetized with urethane (1.2 g/kg, i.p.) and an input–output curve was determined. The current intensity of the test pulses (0.1 ms duration) was set at 40% of the stimulation current that evoked a maximal field population spike. This stimulation intensity was used for test and tetanic stimuli. All the electro-stimuli were constant-current, monophasic square-wave pulses. Thirteen animals were given high-frequency tetanic trains to evoke LTP following a 15-min control baseline recording. An NMDA receptor antagonist, MK801 (5 mg/kg i.p. injection, Research Biochemical International), was preinjected into the other 6 animals, 45 min prior to the first tetanic train. Tetanus consisted of 5 trains of high-frequency stimulation (HFS) (each train, 400 Hz for 1 s, 0.25 ms pulse width) delivered at 2-min inter-train intervals. Both the fEPSP slope and the amplitude of the population spike were monitored as an index of LTP. Test pulse (0.1 ms duration) was delivered at 90-s intervals (low-frequency stimulation, LFS). The evoked responses were amplified, digitized at 50 kHz and stored sequentially on computer discs (NEC PC9801 DX) for off-line analysis. The initial fEPSP slopes were displayed as a percentage of the mean value obtained during the 15 min period just before tetanus. Animals were fixed by cardiac perfusion for in situ hybridization under deep urethane anesthesia either 45 min (HFS, $n=5$; MK801+HFS, $n=2$) or 3 h (HFS, $n=8$; MK801+HFS, $n=4$) after the first train of tetanic stimulation. Six chronically implanted animals, which served as a control, were treated in the same way as the LTP group, except that they did not receive HFS. These animals were fixed 1 h or 3 h 15 min after the recording of the first evoked response ($n=2$ and $n=4$, respectively).

2.3. *In situ hybridization*

Animals injected with PTZ were deeply anesthetized with pentobarbital (50 mg/kg, i.p.). Animals to be examined for the developmental expression profile of *vesl* were anesthetized with diethylether. Anesthetized animals were fixed by cardiac perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4)/saline. In situ hybridization was carried out as described [19] using a DIG-labeling and detection kit (Boehringer Mannheim). DIG-labeled cRNA probe was obtained by in vitro transcription.

2.4. *Miscellaneous methods*

Northern blot was carried out as described [19]. Probe DNA was labeled with [α-^{32}P]dCTP using a random-primer labeling kit (Stratagene). G6PDH cDNA derived from mouse was chosen as a loading control because of its constitutive expression [19]. Plaque hybridization was performed as described [24] with a λZAPII cDNA library constructed from hippocampal RNA of PTZ-treated rats (3 h) [19]. The nucleotide sequence was determined on both strands by the dideoxy chain-termination method using an automatic DNA sequencer.

3. Results

3.1. *Identification of a novel gene induced during neuronal excitation by mRNA differential display*

We previously observed, using PCR differential display, that the levels of a set of cDNAs were increased by neural activity [19,20]. One of the PCR fragments (180 bp), amplified with the pair of primers, T_{12}MT (M, a mixture of A, C and G) and 5′-GGATTCTCAG-3′, was reproducibly induced in the hippocampus after injection of kainate (Fig. 1A). A substantial increase in this PCR product was observed 3 h after the injection of kainate and persisted for at least another 3 h. While the injection of both pentylenetetrazole (PTZ) and cycloheximide (CHX) elicited a moderate increase in the level of the PCR product at 3 h, treatment with PTZ alone did not produce a significant signal at 45 min in this assay system.

The 180 bp fragment, which should correspond to the 3′-end of mRNA, was cloned into the pCRII vector and used for Northern blot analysis as a probe (probe E, Fig. 2A). The Northern blot showed a marked augmentation in the level of this mRNA at 3 and 6 h after kainate injection and a slight increase at 3 h following the injection of PTZ and CHX (Fig. 1B). The mRNA was 6 kb in length and we refer to it as *vesl*. Although no significant change in the levels of *vesl* was detectable 45 min after the PTZ injection, a moderate increase was observed at 45 min when we used a DNA fragment (probe D, 1.9 kb) that was 10 times longer than probe E (data not shown). Thus, the expression of *vesl* mRNA in the hippocampus was strongly induced by kainate treatment

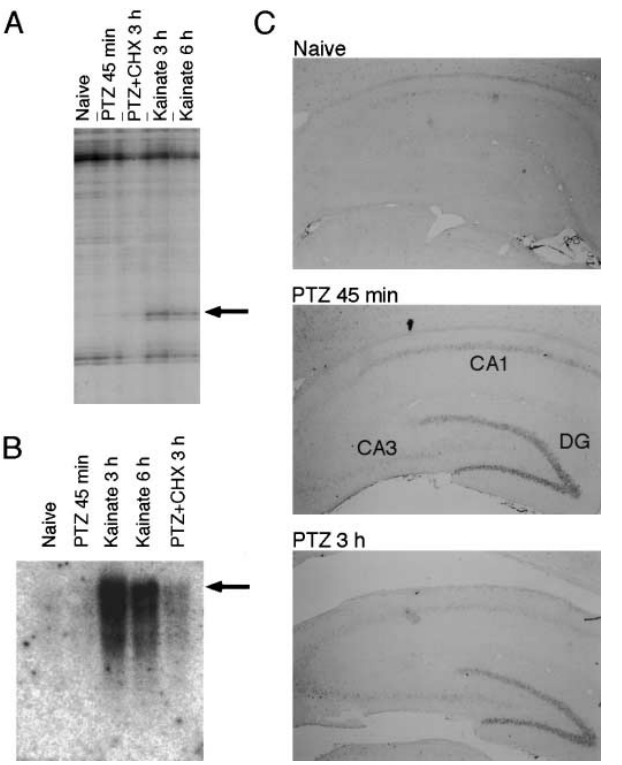

Fig. 1. Increase in the level of *vesl* mRNA following pharmacologically induced convulsive seizure. (A) Autoradiograph of a radiolabeled-PCR differential display with the pair of primers, T_{12}MT and 5′-GGATTCTCAG-3′. Total cellular RNA was isolated from the hippocampal tissue at different times after injection of the drug. An arrow indicates the 180 bp PCR product. (B) Northern blot analysis of the *vesl* mRNA. Five μg of hippocampal total RNA was loaded onto each lane and hybridized with probe E (see Fig. 2A). The same result was obtained when we used probes B and C (not shown). Arrow, *vesl* mRNA of 6 kb. (C) In situ hybridization showing an increase in the *vesl* mRNA in the hippocampus following PTZ administration. DIG-labeled antisense cRNA (probe D, 0.8 μg/μl in hybridization solution) (Fig. 2A) was used.

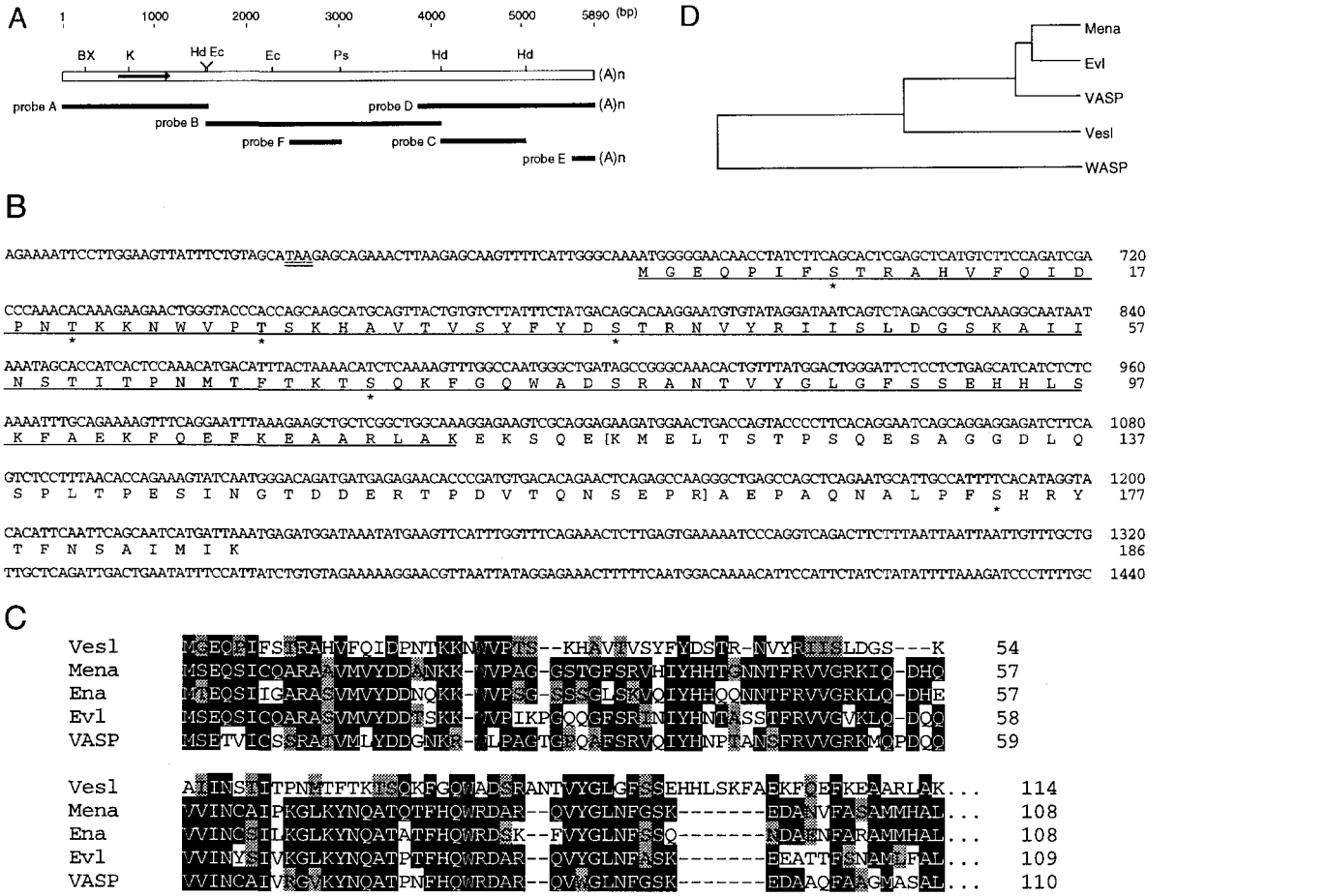

Fig. 2. Primary structure of part of the *vesl* cDNA and comparison of the predicted amino acid sequence with that of the VASP/Mena family of proteins. (A) A physical map of the full-length *vesl* cDNA. Ec, *Eco*RI; BX, *Bst*XI; K, *Kpn*I; Hd, *Hin*dIII; Ps, *Pst*I. An arrow in the box shows the longest ORF. Probes used for Northern analysis and in situ hybridization are also shown. (B) Nucleotide and deduced amino acid sequences of the *vesl* cDNA. In frame-termination codon located upstream of the *vesl* initiation codon is marked by a double underline. The EVH1-like domain is underlined. Putative PKC phosphorylation sites are indicated by asterisks. The conserved PEST sequence is bracketed. The PEST scores calculated with the PEST-find algorithm [30,31] are 11.53 (120–152) and 6.60 (152–163). (C) The amino acid sequence of Vesl was aligned with the following VASP/Ena family members: Mena and Evl [23], Ena [27] and VASP [28]. Amino acid identities between Vesl and other proteins are indicated by white letters in black boxes. Black letters in shadowed boxes represent conserved amino acids. (D) Phylogenetic analysis using the UPGMA method [37] revealed that the EVH1-like domain of Vesl (1–113) falls into a distinct evolutionary subgroup from that of the EVH1 domain of the VASP/Ena family.

and moderately by PTZ, confirming the results of differential display.

The regional and temporal expression pattern of *vesl* mRNA in the hippocampus following PTZ injection was examined more precisely by in situ hybridization (Fig. 1C). Because of higher sensitivity of in situ hybridization compared to Northern blot, the increase in the *vesl* mRNA level was clearly detected in the hippocampus. The induction of *vesl* mRNA was mainly observed in granule cells of the dentate gyrus both 45 min and 3 h after the injection of PTZ, while only very weak induction was seen in the CA1 and CA3 region.

3.2. *Primary structure of vesl mRNA and protein*

A full-length cDNA (5.9 kb in length) was isolated by screening an oligo(dT)-primed cDNA library derived from the hippocampal RNA of PTZ-treated (3 h) rats [19]. The entire nucleotide sequence of the cDNA was determined, and we found the existence of a putative open reading frame (ORF) of 186 amino acids (20871 Da) (Fig. 2). The sequence surrounding the presumptive ATG initiation codon (position 670) was characteristic of the sequence context of eukaryotic initiation codons [25]. The Vesl protein has 6 amino acid residues at positions 8, 20, 27, 40, 71 and 174 which could serve as phosphorylation sites for protein kinase C [26]. A search in the NBRF and Swiss-plot databases revealed that the N-terminal region of Vesl (1–113) has significant homology (26–30% identical amino acids) to the EVH1 domain (Fig. 2C), a highly conserved domain among members of the VASP/Ena family of proteins (60–70% conservation between family members) [23,27,28]. Phylogenetic analysis revealed that the EVH1-like region of Vesl lies between the VASP/Ena family of proteins and Wiskott-Aldrich syndrome protein (WASP) [29] (Fig. 2D). The putative N-terminal methionine of Vesl protein was aligned at the same position as the initiation methionine of the VASP/Ena family proteins (Fig. 2C), supporting that the ATG at position 670 is indeed utilized as a translation initiation codon. PEST score analysis revealed the existence of a conserved PEST sequence in the C-terminal region (Fig. 2B). The PEST sequence is found in proteins with high turnover rates and has been postulated to be involved in

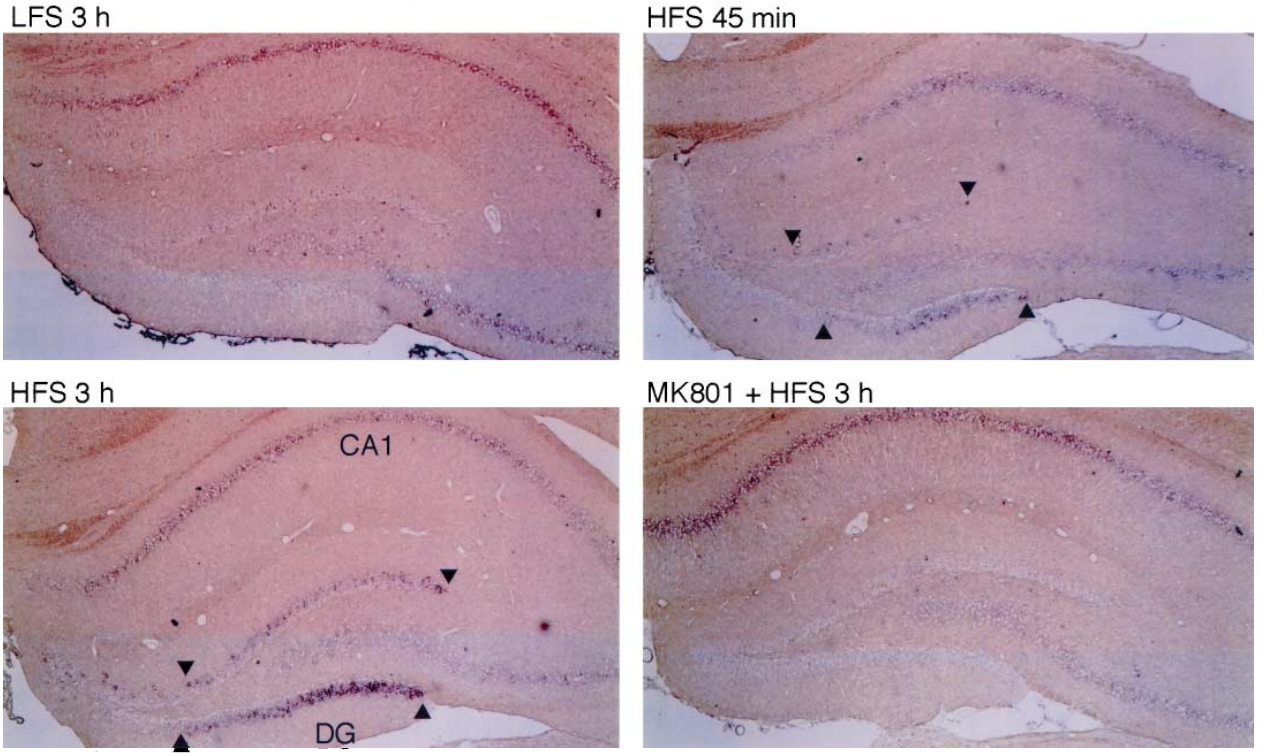

Fig. 3. HFS delivered to the perforant path induced *vesl* mRNA expression in the granule cells. Typical photomicrographs of in situ hybridization are shown. DIG-labeled *vesl* cRNA probe (probe A, 12.5 μg/μl in hybridization solution) was hybridized to sections of the dorsal hippocampus following LFS (3 h), HFS (45 min), HFS (3 h), or MK801+HFS (3 h) (MK801 was injected 30 min prior to HFS). DG, dentate gyrus; CA1, CA1 field of the hippocampus. The increase in the level of *vesl* mRNA is observed in the regions delimited by triangles. The same result was obtained with probe D (not shown).

protein degradation [30,31]. Therefore, the Vesl protein is likely to consist of two functional domains, the EVH1 homologous region at the N-terminus and a putative degradation signal at the C-terminus.

3.3. *vesl mRNA was induced during LTP*

We next investigated whether the expression of *vesl* mRNA is regulated during plastic changes in synaptic efficacy. Under urethane anesthesia, 5 trains of HFS (400 Hz for 1 s) were given to the perforant pathway to elicit LTP in the dentate gyrus. This tetanic stimulation usually produced a long-lasting enhancement of synaptic transmission measured from the dentate gyrus either as the fEPSP slope or as the amplitude of the population spike (>10 h) (Inokuchi, unpublished observation). Delivery of this tetanic stimulation caused an increase in *vesl* mRNA after 45 min ($n=5$) in the dentate gyrus ipsilateral to the stimulation electrode (Fig. 3). The increase was maintained at least for 3 h ($n=6$). In this experiment, we used a higher concentration of cRNA probe (12.5 μg/μl) compared to Fig. 1C (0.8 μg/μl). Thus, basal level of *vesl* mRNA expression in the CA1 region, which was not observed in Fig. 1C, could be detected (see also Fig. 4). We found no differences in the expression profile between LFS control and naive rats (compare Figs. 3 and 4), indicating that the chronic implantation of electrode did not affect the expression of *vesl* mRNA. Northern blot analysis using probe F showed an increase in the level of *vesl* mRNA 3 h after the induction of LTP (not shown).

Among 13 animals that had received the same trains of HFS, 2 animals failed to maintain synaptic potentiation for unknown reasons. In one case, delivery of HFS caused a transient enhancement both in the fEPSP slope and the amplitude of population spike. However, the enhancement returned to the baseline level approximately 2 h after HFS. In the other case, we did not observe LTP. Analysis of these 2 animals using in situ hybridization showed no obvious *vesl* signals in the dentate granule cells at 3 h (data not shown). Furthermore, administration of the NMDA receptor antagonist MK801, which completely prevented the production of LTP, also blocked completely the increase in *vesl* mRNA in the granule cells ($n=4$) (Fig. 3, see also Fig. 5). These results indicate that the induction of *vesl* expression by HFS correlates well with the persistence of LTP, and that the expression of *vesl* mRNA is induced in an NMDA receptor-dependent manner in the dentate granule cells.

3.4. *Developmental regulation of vesl mRNA expression in the rat hippocampus*

We next addressed the question of whether the *vesl* mRNA is expressed during the period of neurogenesis and/or synaptogenesis. The expression profile of *vesl* mRNA in the rat hippocampus from embryonic day 20 (E20) to the adult was examined by in situ hybridization (Fig. 4). At E20, a slight *vesl* mRNA signal was located in the subicular area. At P7, the level of *vesl* mRNA became prominent in the CA1–4 regions and a weak signal was observed in the dentate gyrus. At P14, the intensity of the hybridization signal peaked in the CA1–4 regions, while the weak signal in the dentate gyrus showed no appreciable change compared to P7. In the hilus region, most of the scattered, large cells, which may be migrating precursors of dentate granule cells, showed moderate hybridization signals. By P21, the expression level in the pyramidal cell layer

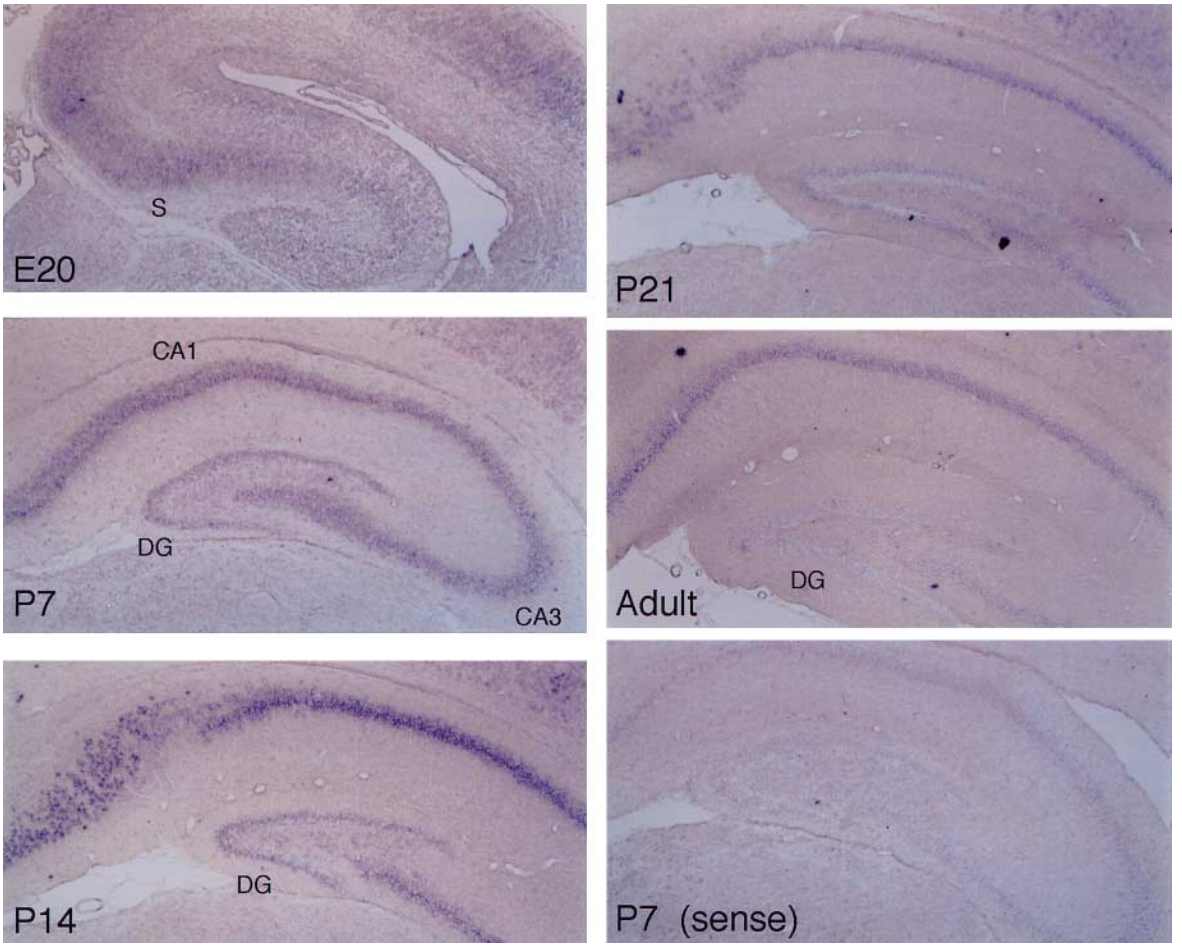

Fig. 4. Expression of the *vesl* mRNA in the developing hippocampus. Coronal sections of the brain dissected from E20, P7, P14, P21 and adult animals were hybridized with a DIG-labeled antisense *vesl* cRNA probe (probe A, 12.5 μg/μl in hybridization solution). Sense cRNA probe of the same concentration was used as a control. DG, dentate gyrus; CA1, CA1 field of the hippocampus; S, subiculum.

had decreased to the adult level. In the dentate gyrus, the weak signal was restricted to the ectal blade. In young adults (14 weeks), *vesl*-specific labeling of the dentate gyrus had faded out to the background level. Thus, the increase in the *vesl* mRNA in the dentate gyrus appears to correspond to a particular stage of development, termed synaptogenesis [32]. These results suggest an important role for Vesl protein in the de novo formation of the synapse.

3.5. Tissue distribution of vesl expression in the adult rat

Northern blot analysis showed absence of significant hybridization signals for *vesl* in non-neuronal tissues such as thymus, lung, heart, liver, spleen, intestine, kidney, testis, and skeletal muscle of the adult rat (Fig. 5).

4. Discussion

Here we have described a novel gene, *vesl*, the expression of which is enhanced by a pharmacologically induced seizure in the hippocampus. This induction was observed even in the presence of CHX. Therefore, *vesl* represents one of the immediate-early genes. The increase in the expression of transcripts was also observed during a persistent LTP in the dentate granule cells. Preinfusion of MK801 blocked the HFS-induced increase in expression, indicating that membrane depolarization per se is not sufficient for *vesl* induction. Rather, the induction of the *vesl* mRNA was closely associated with activity-dependent synaptic plasticity in the adult hippocampus.

The N-terminus of the Vesl protein displays significant ho-

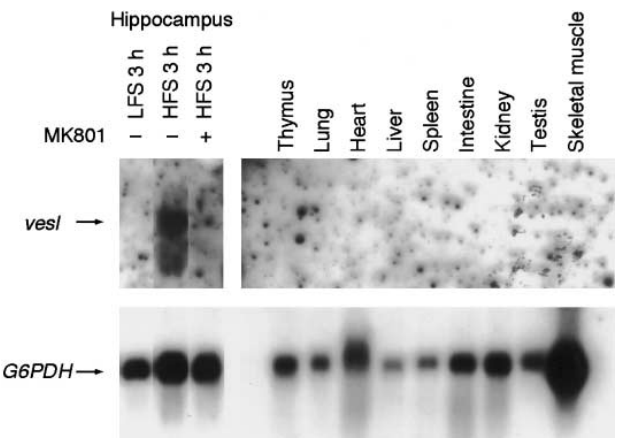

Fig. 5. The expression of *vesl* is mainly observed in the LTP-induced hippocampus but not in the other tissues. Northern analysis of total RNA prepared from hippocampus and from peripheral tissues. ^{32}P-labeled cDNA (probe A) was used for hybridization. The same blots were re-hybridized with the G6PDH probe, which served as a loading control. Note that a high background in the top panel of the autoradiogram is due to long exposure time (top panel, 24 h; bottom, 1 h).

mology to the EVH1 domain of the VASP/Ena family of proteins. Mena, the mammalian homologue of *Drosophila Enabled* (*Ena*), and VASP are localized at focal contacts in a fibroblast cell line and are concentrated in the growth cones of the neuron, a highly motile structure at the end of growing axons and dendrites [22,23]. Ena is required for proper assembly of axonal structures. The EVH1 domain of Mena directly binds to zyxin [23], a LIM domain protein that has a role in coordinating membrane and cytoskeletal interactions [33]. The interaction between Mena and zyxin is important for Mena localization at focal contacts [23]. Therefore, it is likely that the N-terminal region of Vesl, which comprises an EVH1-like domain, targets Vesl to focal contacts and, perhaps, to growth cones in neuronal cells. The EVH1 domain of VASP/Ena family proteins is followed by a proline-rich region that serves as a profilin-binding motif. Profilin is an actin-monomer-binding protein that promotes the formation of F-actin [34,35]. By this means, VASP/Ena family proteins function as a structural linkage between cytoskeletal proteins, such as actin, and the extracellular matrix. Importantly, ectopic expression of the neural-enriched isoform of Mena in a fibroblast cell line induces the formation of protuberances enriched in F-actin from the cell surface [23]. This implies a role for Mena in actin-based extension of filopodia during axonal growth cone migration in neuronal cells.

Instead of a proline-rich motif, Vesl contains a PEST sequence associated with the EVH1-like domain. Two fundamental characteristics of the Vesl protein, i.e. its inducibility during hippocampal LTP and domain structure comprising an EVH1-like domain at the N-terminus and a rapid degradation signal at the C-terminus, suggest that it might compete with other protein(s) that are expressed constitutively and engaged in cytoskeletal dynamics. In fact, we have recently isolated a novel cDNA encoding a protein that contains an EVH1-like domain closely related to that of Vesl and a longer C-terminal portion (Kato, Ozawa and Inokuchi, unpublished results). We suggest that Vesl may be a dominant negative regulator for a component of the adhesion/cytoskeleton apparatus, thereby modulating the cytoskeletal dynamics of growth cones in response to stimuli that elicits a persistent LTP.

In neuronal cells, the direction and the pattern of neurite growth are determined by the growth cones. The motility of the growth cones largely depends on the actin-based structures, filopodia and lamellipodia. Vesl may function as a key effector component that links de novo mRNA and protein synthesis, required for the maintenance of LTP, to the morphological changes at synaptic connections, including synapse restructuring and increase in the total number of synaptic contacts that accompany the maintenance phase of LTP [36].

During development of the hippocampus, the expression of the *vesl* transcript reached a maximum during postnatal weeks 2–3, the period during which neuronal differentiation and synaptogenesis extensively occur in the hippocampus [32]. Thus, the enhancement of *vesl* expression was observed not only during artificially evoked plastic change in synaptic transmission, but also during the developmental construction of the neural network. In the developmental stage, neurite growth and formation of synapses are substantial steps in neuronal differentiation and in the construction of the neural network in the brain. It is quite possible to envisage that, in some cases, the molecular mechanisms required for neuronal plasticity in adulthood are the same as those involved in network formation during the developmental stage. If this were the case, then Vesl would play a pivotal role in such a mechanism by co-ordinating the construction of neural circuits during development and by reorganising neural circuits during the acquisition of new information.

Acknowledgements: We wish to thank Maki Inoue for her helpful advice on LTP experiments in vivo, and Fumio Hishinuma for suggestions on the PEST sequence analysis.

References

[1] Inokuchi, K., Kato, A., Hirai, K. and Ozawa, F. (1996) Soc. Neurosci. Abstr. 22, 1514.
[2] Brakeman, 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.
[3] Davis, H.P. and Squire, L.R. (1984) Psychol. Bull. 96, 518–559.
[4] Goelet, P., Castellucci, V.F., Schacher, S. and Kandel, E.R. (1986) Nature 322, 419–422.
[5] Bliss, T.V.P. and Collingridge, G.L. (1993) Nature 361, 31–39.
[6] Frey, U., Krug, M., Reymann, K.G. and Matthies, H. (1988) Brain Res. 452, 57–65.
[7] Otani, S., Marshall, C.J., Tate, W.P., Goddard, G.V. and Abraham, W.C. (1989) Neuroscience 28, 519–526.
[8] Abraham, W.C., Mason, S.E., Demmer, J., Williams, J.M., Richardson, C.L., Tate, W.P., Lawlor, P.A. and Dragunow, M. (1993) Neuroscience 56, 717–727.
[9] Frey, U., Huang, Y.Y. and Kandel, E.R. (1993) Science 260, 1661–1664.
[10] Nguyen, P.V., Abel, T. and Kandel, E.R. (1994) Science 265, 1104–1107.
[11] Cole, A.J., Saffen, D.W., Baraban, J.M. and Worley, P.F. (1989) Nature 340, 474–476.
[12] Wisden, W., Errington, M.L., Williams, S., Dunnett, S.B., Waters, C., Hitchcock, D., Evan, G., Bliss, T.V.P. and Hunt, S.P. (1990) Neuron 4, 603–614.
[13] Qian, Z., Gilbert, M.E., Colicos, M.A., Kandel, E.R. and Kuhl, D. (1993) Nature 361, 453–457.
[14] Nedivi, E., Hevroni, D., Naot, D., Israeli, D. and Citri, Y. (1993) Nature 363, 718–721.
[15] Yamagata, K., Andreasson, K.I., Kaufmann, W.E., Barnes, C.A. and Worley, P.F. (1993) Neuron 11, 371–386.
[16] Yamagata, K., Kaufmann, W.E., Lanahan, A., Papapavlou, M., Barnes, C.A., Andreasson, K.I. and Worley, P.F. (1994) Learning Memory 1, 140–152.
[17] Link, W., Konietzko, U., Kauselmann, G., Krug, M., Schwanke, B., Frey, U. and Kuhl, D. (1995) Proc. Natl. Acad. Sci. USA 92, 5734–5738.
[18] Lyford, G.L., Yamagata, K., Kaufmann, W.E., Barnes, C.A., Sanders, L.K., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Lanahan, A.A. and Worley, P.F. (1995) Neuron 14, 433–445.
[19] Inokuchi, K., Kato, A., Hirai, K., Hishinuma, F., Inoue, M. and Ozawa, F. (1996) FEBS Lett. 382, 48–52.
[20] Inokuchi, K., Murayama, A. and Ozawa, F. (1996) Biochem. Biophys. Res. Commun. 221, 430–436.
[21] Andreasson, K. and Worley, P.F. (1995) Neuroscience 69, 781–796.
[22] Reinhard, M., Halbrugge, M., Scheer, U., Wiegand, C., Jockusch, B.M. and Walter, U. (1992) EMBO J. 11, 2063–2070.
[23] Gertler, F.B., Niebuhr, K., Reinhard, M., Wehland, J. and Soriano, P. (1996) Cell 87, 227–239.
[24] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.
[25] Kozak, M. (1987) Nucleic Acids Res. 15, 8125–8148.
[26] Kenelly, P.J. and Krebs, E.G. (1991) J. Biol. Chem. 266, 15555–15558.
[27] Gertler, F.B., Comer, A.R., Juang, J.L., Ahern, S.M., Clark, M.J., Liebl, E.C. and Hoffmann, F.M. (1995) Genes Dev. 9, 521–533.
[28] Haffner, C., Jarchau, T., Reinhard, M., Hoppe, J., Lohmann, S.M. and Walter, U. (1995) EMBO J. 14, 19–27.

[29] Miki, H., Miura, K. and Takenawa, T. (1996) EMBO J. 15, 5326–5335.
[30] Rogers, S., Wells, R. and Rechsteiner, M. (1986) Science 234, 364–368.
[31] Rechsteiner, M. and Rogers, S.W. (1996) Trends Biochem. Sci. 21, 267–271.
[32] Gaarskjaer, F.B. (1985) J. Comp. Neurol. 241, 154–170.
[33] Crawford, A.W., Michelsen, J.W. and Beckerle, M.C. (1992) J. Cell Biol. 116, 1381–1393.
[34] Pantaloni, D. and Carlier, M.F. (1993) Cell 75, 1007–1014.
[35] Theriot, J.A. and Mitchison, T.J. (1993) Cell 75, 835–838.
[36] Geinisman, Y., Detoledo, M.L., Morrell, F., Persina, I.S. and Beatty, M.A. (1996) J. Comp. Neurol. 368, 413–423.
[37] Sokal, R.R.a.M., C.D. (1958) Univ. Kansas Sci. Bull. 28, 1409–1438.