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Journal of Chemical Neuroanatomy 30 (2005) 201–211
Journal of CHEMICAL NEUROANATOMY
www.elsevier.com/locate/jchemneu

# Distribution of synaptobrevin/VAMP 1 and 2 in rat brain

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Received 30 November 2004; received in revised form 1 August 2005; accepted 1 August 2005
Available online 16 September 2005

**Abstract**

The synaptobrevin/vesicle-associated membrane protein (VAMP) family of proteins, which are essential for neurotransmitter release, are the vesicle donor soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor (SNARE) proteins first described in synaptic vesicles at nerve terminals.

Two synaptobrevin/VAMP isoforms are involved in calcium-dependent synaptic vesicle exocytosis, synaptobrevin/VAMP 1 and synaptobrevin/VAMP 2. However, the functional significance of these two highly homologous isoforms remains to be elucidated.

Here, we used immunohistochemical, immunofluorescence and confocal microscope techniques to localize the two synaptobrevin/VAMP isoforms in rat brain areas, particularly in nerve terminals. Our results show that the two isoforms are present in the rat central nervous system and that their expression overlaps in some areas. However, a distinct distribution pattern was detected. Synaptobrevin/VAMP 2 is the most abundant isoform in the rat brain and is widely distributed. Although synaptobrevin/VAMP 1 is less abundant, it is the main isoform in particular brain areas (e.g. zona incerta at the subthalamus or nerve terminals surrounding thalamic neurons). The colocalization of synaptophysin with synaptobrevin/VAMP 1 demonstrates the presence of this isoform in subsets of nerve terminals. These results indicate that each synaptic vesicle donor SNARE protein isoform could have a specialized role in the neurosecretory process.
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*Keywords:* SNARE proteins; Hippocampus; Syntaxin; SNAP25; Immunofluorescence

## 1. Introduction

The late steps of synaptic vesicle exocytosis are regulated by a set of proteins that form a complex directly involved in the fusion of synaptic vesicles with the presynaptic plasma membrane. The core of the complex comprises four protein motifs or soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor (SNARE) motifs. One of these belongs to a protein located in the vesicle donor compartment. This protein has an arginine residue in the central ionic layer (v-, vesicle; r-, arginine SNARE) that forms hydrogen bonds with three glutamine residues, each from one of the three remaining SNARE motifs. These three motifs are distributed in two target plasma membrane proteins located in the acceptor compartment (t-, target; q-, glutamine SNARE; Jahn and Sudhof, 1999).

The v- or r-SNARE involved in synaptic vesicle exocytosis is the synaptic *v*esicle-*a*ssociated *m*embrane *p*rotein synaptobrevin/VAMP while the two t- or q-SNARE plasma membrane-associated proteins are syntaxin 1 and SNAP-25, with one and two SNARE motifs, respectively (Sollner et al., 1993; Sutton et al., 1998). SNARE motifs mediate the pairing of syntaxin 1, SNAP25 and synaptobrevin/VAMP, leading to the formation of the protein complex (SNARE complex), which provides the scaffold for the subsequent calcium-dependent fusion of membrane compartments, the synaptic vesicles and the presynaptic plasma membrane.

Curiously, in the mammalian nervous system, synapses contain two isoforms of each of the SNARE proteins originally reported in nerve terminals. Concretely, two synaptobrevin/VAMP isoforms were initially identified as synaptic proteins, synaptobrevin/VAMP 1 and synaptobrevin/VAMP

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doi:10.1016/j.jchemneu.2005.08.002

2, and were later found in non-neuronal cell types with regulated exocytosis (Elferink et al., 1989; Rossetto et al., 1996). Synaptobrevin/VAMP is a short, very abundant synaptic vesicle protein with a central SNARE motif, a carboxy terminal transmembrane region and a proline-rich amino terminus that is not well conserved between species and that, together with the hydrophobic carboxy terminus, contains most of the amino acid differences between synaptobrevin/VAMP 1 and synaptobrevin/VAMP 2 (Elferink et al., 1989; Trimble et al., 1990).

Although SNARE proteins show promiscuous interaction in vitro, these interactions have not been observed in vivo, where SNARE complexes are well defined and the putative functional significance of cross-interaction between elements of distinct complexes remains obscure. Although the presence of two isoforms in the same cell and tissue may imply a redundant system for cell survival, assuming the isoforms share identical functions, it has been clearly demonstrated using synaptobrevin/VAMP 2 knockout mice that at least this isoform is consistently required for organism survival (Schoch et al., 2001). Still it is not known why more than one synaptobrevin/VAMP isoform is present in the same cellular structure. However, several differences in their interaction affinity to form SNARE complexes in vitro (Perez-Branguli et al., 1999, 2002) and their differential expression in the central and peripheral nervous system indicate that the two isoforms have specialized functions (Trimble et al., 1990; Rossetto et al., 1996; Jacobsson et al., 1998). These observations reflect heterogeneity in the composition of synaptic vesicles, nerve endings and even neurons, on the basis of synaptobrevin/VAMP isoform content.

Previous studies on rat brain using in situ hybridization showed a differential distribution of neurons expressing synaptobrevin/VAMP isoforms. Synaptobrevin/VAMP 2 was more evenly distributed while synaptobrevin/VAMP 1 expression was located mainly in neurons with somatomotor function (Trimble et al., 1990). In addition, a differential distribution of these two isoforms occurs in the mouse retina (Sherry et al., 2003). However, few studies have addressed the distribution of synaptobrevin/VAMP isoform proteins in brain regions.

Here, we examined the distribution of synaptobrevin/VAMP 1 and 2 in the rat brain by means of specific isoform antibodies. Our results show a differential distribution in some areas of the rat brain, with partial overlapping, and suggest specialized functional roles for each isoform.

## 2. Materials and methods

### 2.1. Antibodies and serum purification

A commercially available monoclonal antibody was used against synaptobrevin/VAMP 2 (clone 69.1, Synaptic Systems). A rabbit antiserum against synaptobrevin/VAMP 1 was generated in our laboratory using a cytosolic fragment of recombinant synaptobrevin/VAMP 1 as antigen. To avoid synaptobrevin/VAMP 1 antiserum cross reactivity with isoform 2, the antiserum was previously absorbed with synaptobrevin/VAMP 2–GST bound to glutathione–sepharose beads.

Cytosolic fragments of synaptobrevin/VAMP 1 and 2 cloned into pGEX expression vector (Pharmacia Biotech) were expressed overnight at room temperature (RT) as glutathione *S*-transferase (GST) fusion proteins after 0.4 mM of isopropyl β-D-thiogalactopyranoside (IPTG, Sigma) induction. Cells were centrifuged at 6000 rpm for 20 min in a Beckman JA-20 rotor and pellets were resuspended in ice-cold phosphate buffered saline (PBS) with 1% Triton X-100, 2 mM EGTA, 2 mM EDTA and protease inhibitors (0.5 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin) and frozen at −20 °C until used. Samples were thawed and a cell extract was prepared after sonication and centrifugation at 12,000 rpm for 20 min in a JA20 Beckman rotor at 4 °C. The resulting supernatants were incubated overnight at 4 °C with glutathione–sepharose beads (Pharmacia Biotech) previously equilibrated in PBS. After incubation, beads were washed with PBS and kept in the same buffer (with protease inhibitors as above) at 4 °C until use. The purity of recombinant fusion proteins was analyzed by Coomassie Brilliant Blue stained SDS–PAGE.

Synaptobrevin/VAMP isoform antibodies were analyzed by Western blotting with synaptosomal extract from rat brain. Synaptosomes were loaded in a 14% acrylamide-bisacrylamide gel. After SDS–PAGE, proteins were transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA). These were blocked with 5% non-fat dry milk in Tris-buffered saline (TBS) (140 mM NaCl, 10 mM Tris–HCl pH 7.4 with 0.1% Tween-20) for 45 min at RT, and incubated with anti-synaptobrevin/VAMP 1 serum (1:500 dilution) and monoclonal anti-synaptobrevin/VAMP 2 (1:2,500 dilution) overnight at 4 °C. After three washes in the same buffer, membranes were incubated with anti-rabbit or anti-mouse immunoglobulins conjugated to horseradish peroxidase (DAKO). Finally, after five washes with TBS, the blots were developed using the enhanced chemiluminescence method (ECL).

Isoform specificity of these synaptobrevin/VAMP antibodies was tested by Western blot using 1 μg of recombinant synaptobrevin/VAMP 1 and 2, following the procedure above.

To rule out cross reactivity of synaptobrevin/VAMP 1 antiserum with other members of the synaptobrevin/VAMP family, extracts of the insulinoma cell line INS-1, which contains synaptobrevin/VAMP 2 and 3 (also called cellubrevin), were tested by Western blot. The INS-1 cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) FBS, 10 mM HEPES, 2 mM glutamine, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 100 U/ml penicillin, 100 U/ml streptomycin and 11 mM glucose. Cells were grown, washed with PBS, scrapped, pelleted and homogenated in PBS with 1% Triton X-100 and protease

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