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REVIEW

EXPRESSION AND CELLULAR FUNCTION OF vSNARE PROTEINS IN BRAIN ASTROCYTES

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Abstract—Gray matter protoplasmic astrocytes, a major type of glial cell in the mammalian brain, extend thin processes ensheathing neuronal synaptic terminals. Albeit electrically silent, astrocytes respond to neuronal activity with Ca^{2+} signals that trigger the release of gliotransmitters, such as glutamate, D-serine, and ATP, which modulate synaptic transmission. It has been suggested that the astrocytic processes, together with neuronal pre- and post-synaptic elements, constitute a tripartite synapse, and that astrocytes actively regulate information processing. Astrocytic vesicles expressing VAMP2 and VAMP3 vesicular SNARE (vSNARE) proteins have been suggested to be a key feature of the tripartite synapse and mediate gliotransmitter release through Ca^{2+}-regulated exocytosis. However, the concept of exocytotic release of gliotransmitters by astrocytes has been challenged. Here we review studies investigating the expression profile of VAMP2 and VAMP3 vSNARE proteins in rodent astrocytes, and the functional implication of VAMP2/VAMP3 vesicles in astrocyte signaling. We also discuss our recent data suggesting that astrocytic VAMP3 vesicles regulate the trafficking of glutamate transporters at the plasma membrane and glutamate uptake. A better understanding of the functional consequences of the astrocytic vSNARE vesicles on glutamate signaling, neuronal excitability and plasticity, will require the development of new strategies to selectively interrogate the astrocytic vesicles trafficking *in vivo*.

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Abbreviations: AQP4, aquaporin 4 water channel; BoNT, botulinum toxin; dnSNARE, dominant negative SNARE; EM, electron microscopy; GLAST, glutamate aspartate transporter; GLT-1, glutamate transporter 1; GPCR, G protein-coupled receptor; KO, knockout; miniSOG, mini singlet oxygen generator; NSF, N-ethylmaleimide-sensitive fusion protein; SNAP, soluble NSF attachment protein; SNARE, soluble NSF attachment protein receptor; TeNT, tetanus toxin; Tg, transgenic; VAMP, vesicle-associated membrane protein; vSNARE, vesicle-associated SNARE; WT, wild type.

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Contents

INTRODUCTION

The gray matter protoplasmic astrocytes (thereafter called astrocytes) are a major type of glial cells in the mammalian brain (Barres, 2008). They have a small cell body and extend poorly ramified processes with their endfeet contributing to the formation of the blood–brain barrier (reviewed by Abbott et al., 2006), and highly ramified bushy processes ensheathing neuronal pre- and post-synaptic elements (Ventura and Harris, 1999; Halassa et al., 2007; Korogod et al., 2015). Astrocytes are electrically non-excitable cells, and it has been classically assumed that they do not contribute to information processing, but rather control the *milieu intérieur* by buffering the potassium ions, and regulating cell volume, amino acid uptake, and metabolic supplies in response to neuronal activity (reviewed by Kimelberg, 2010; Nagelhus and Ottersen, 2013; Zhou and Danbolt, 2013; Magistretti and Allaman, 2015). Thereby, astrocytes support neuronal survival. Interestingly, the homeostatic functions of astrocytes can also regulate neuronal activity. For example, *in vivo* inactivation of glutamate transport leads to changes in synaptic transmission, and to chronic

hyperexcitability with behavioral deficits (Cui et al., 2014; Aida et al., 2015; Petr et al., 2015). Also, glucose supply by astrocytes supports synaptic transmission (Rouach et al., 2008).

In the late 1990s, it has been suggested that astrocytes are not only supportive cells and the concept of a tripartite synapse has been developed (reviewed by Araque et al., 1999; Araque et al., 2014). Using cultured astrocytes (Bezzi et al., 2004; Mothet et al., 2005; Pangrsic et al., 2007), brain slices (Zhang et al., 2003; Perea and Araque, 2005; Henneberger et al., 2010), and *in vivo* preparations (Halassa et al., 2009; Navarrete et al., 2012), it was shown that astrocytes respond to neuronal activity with Ca^{2+} signals that trigger the release of gliotransmitters, such as glutamate, ATP, GABA, and D-serine, which in turn modulate synaptic transmission and plasticity. It was suggested that the release of gliotransmitters exerts a spatially confined, time-locked feedback control of nearby synapses, much like their neuronal counterparts. One key aspect of the tripartite synapse is the release of gliotransmitters via Ca^{2+}- and soluble NSF attachment protein receptor (SNARE) protein-dependent exocytosis as suggested using cultured astrocytes (Bezzi et al., 2004; Domercq et al., 2006; Jourdain et al., 2007; Marchaland et al., 2008). However, recent experiments with brain slices raised concerns about the ability of astrocytes to release gliotransmitters (Fiacco et al., 2007; Agulhon et al., 2010). In cultured astrocytes and brain slices, it has also been shown that astrocytes release gliotransmitters through non-exocytotic pathways, such as volume-regulated anion channels (Takano et al., 2005; Li et al., 2012), and mouse bestrophin-1 channel (Lee et al., 2010; Woo et al., 2012).

EXPRESSION OF VSNARE VAMP2 AND VAMP3 PROTEINS IN ASTROCYTES

Vesicular fusion is a ubiquitous cellular phenomenon that depends on SNARE proteins. Vesicular fusion mediates: (i) transport of vesicular cargo and membrane proteins inside cells (Jurado et al., 2013); (ii) release of vesicular cargo to extracellular medium after vesicular fusion with plasma membrane (Schoch et al., 2001); and (iii) membrane repair (Rao et al., 2004). Mammals express many SNARE proteins (38 in humans) forming a α-helical ternary complex that mediates vesicle fusion (Weber et al., 1998). The molecular diversity of SNARE complexes is thought to contribute to the functional diversity of exocytotic events (Zorman et al., 2014).

The best characterized SNARE complex in terms of structure and function is found at neuronal synapses where it mediates fast (<0.2 ms) Ca^{2+}-regulated exocytosis of neurotransmitters. Neuronal synaptic SNARE proteins include vesicle-associated SNARE (vSNARE) protein VAMP2 (synaptobrevin 2), and the plasma membrane tSNARE proteins, syntaxin 1 and SNAP-25 (reviewed by Jahn and Fasshauer, 2012; Kasai et al., 2012). VAMP2 is abundantly expressed on neuronal synaptic vesicles (Takamori et al., 2006), and its genetic inactivation leads to a loss of Ca^{2+}-regulated neurotransmitter release (Schoch et al., 2001).

What made the study of synaptic SNARE proteins dynamic and fruitful was the possibility to record synaptic activity in a reliable manner, and to correlate synaptic activity with SNARE protein expression. Gliotransmission is a rare event with slow kinetics; therefore it is more difficult to work out its molecular machinery. In astrocytes, a first issue concerns the expression profile of vSNARE proteins. Although there appears to be some consensus about expression of the VAMP3 vSNARE protein by astrocytes, data on VAMP2 expression by astrocytes from brain slices (Chilcote et al., 1995; Bezzi et al., 2004; Wilhelm et al., 2004; Bergami et al., 2008; Schubert et al., 2011), acutely dissociated astrocytes (Zhang et al., 2004), and cultured astrocytes (Montana et al., 2004; Crippa et al., 2006; Martineau et al., 2008; Bergami et al., 2008; Liu et al., 2011) are controversial.

In a recent study (Li et al., 2015), we compared VAMP2 and VAMP3 expression in cultured astrocytes from wild-type (WT), heterozygous, and VAMP2 or VAMP3 knockout (KO) littermates using fluorescence immunostaining. We found that mouse cortical astrocytes in culture express VAMP3 but not VAMP2. The next step will be to validate this finding *in situ*, and to identify vesicular structures carrying VAMP3. As a preliminary experiment, we performed multi-color immunostaining against VAMP2, and neuronal or astrocytic markers in cortical slices from adult WT mice. Using the quantitative colocalization analysis toolbox (Li et al., 2013), we confirmed neuronal expression of VAMP2, but we found no evidence for VAMP2 expression by astrocytes (Fig. 1), confirming previous data in rodent hippocampal slices (Chilcote et al., 1995; Bezzi et al., 2004; Schubert et al., 2011), and transcriptome data showing that VAMP3 gene expression is enriched in astrocytes as compared to neurons, while VAMP2 is selectively enriched in neurons (Cahoy et al., 2008; Zhang et al., 2014). Due to the diffraction-limited resolution (~200 nm) of light confocal microscopy, further investigations using electron microscopy (EM) as well as WT and VAMP2 KO littermates will help to provide more information about cellular and subcellular distribution of VAMP2 *in situ*. The validation of VAMP3 expression by cultured astrocytes (Li et al., 2015) is in line with previous results *in situ* (Bezzi et al., 2004; Wilhelm et al., 2004; Schubert et al., 2011). Adding control experiments with VAMP3 KO animals will help to further validate and quantify the VAMP3 expression level in astrocytes as compared with other cell types. To overcome the low-efficiency and variability of endogenous protein labeling in EM preparations with antibodies, an alternative approach could be to knock in the VAMP2 or VAMP3 genomic locus with newly developed genetically encoded EM probes such as mini singlet oxygen generator (miniSOG), enhanced ascorbate peroxidase (APEX), and spaghetti monster fluorescent proteins (smFPs) (Shu et al., 2011; Lam et al., 2015; Viswanathan et al., 2015), to obtain high-sensitivity delineation of endogenous protein expression.