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ASTROCYTIC VESICLES AND GLIOTRANSMITTERS: SLOWNESS OF VESICULAR RELEASE AND SYNAPTOBREVIN2-LADEN VESICLE NANOARCHITECTURE

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Abstract—Neurotransmitters released at synapses activate neighboring astrocytes, which in turn, modulate neuronal activity by the release of diverse neuroactive substances that include classical neurotransmitters such as glutamate, GABA or ATP. Neuroactive substances are released from astrocytes through several distinct molecular mechanisms, for example, by diffusion through membrane channels, by translocation via plasmalemmal transporters or by vesicular exocytosis. Vesicular release regulated by a stimulus-mediated increase in cytosolic calcium involves soluble N-ethyl maleimide-sensitive fusion protein attachment protein receptor (SNARE)-dependent merger of the vesicle membrane with the plasmalemma. Up to 25 molecules of synaptobrevin 2 (Sb2), a SNARE complex protein, reside at a single astroglial vesicle; an individual neuronal, i.e. synaptic, vesicle contains ~70 Sb2 molecules. It is proposed that this paucity of Sb2 molecules in astrocytic vesicles may determine the slow secretion. In the present essay we shall overview multiple aspects of vesicular architecture and types of vesicles based on their cargo and dynamics in astroglial cells.

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Key words: exocytosis, vesicles, gliotransmission, astrocytes, ATP, glutamate.

INTRODUCTION

The concept of neuroglia as the connective tissue of the central nervous system (CNS) was introduced in the middle of the 19th century by Rudolf Virchow (Virchow, 1858; Kettenmann and Verkhratsky, 2008). Numerous hypotheses of the neuroglial function were developed by the beginning of the 20th century (Schleich, 1894; Ramón y Cajal, 1895; Golgi, 1903; Lugaro, 1907), which envisaged the role of these cells in modulating synaptic transmission, supporting brain metabolism, controlling blood flow and regulating sleep behavior.

In 1910 Jean Nageotte suggested, based on his microscopic observations, that glial cells (astroglia in particular) act as secretory elements of the CNS (Nageotte, 1910). This hypothesis had been experimentally confirmed in the last quarter of the 20th century, when it was discovered that not only neurons, but also astrocytes, release chemical transmitters (generally known as gliotransmitters, merely to indicate the cellular source of these compounds), which signal to the neighboring cells (this signaling being termed "gliotransmission"). The concept of gliotransmission emerged after the discovery that cultured astrocytes responded to the neurotransmitters, such as glutamate, by generating cytosolic calcium signals (Cornell-Bell et al., 1990) and thereby exhibiting an ability to "sense" glutamatergic synaptic transmission (Dani et al., 1992). This was followed by the discovery that astrocytic calcium dynamics can trigger astrocyte-neuron signaling, with at least two underlying mechanisms: direct, perhaps using gap junctions (Nedergaard, 1994), and indirect utilizing glutamate

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Abbreviations: ANP, atrial natriuretic peptide; $[Ca^{2+}]_i$, cytosolic calcium concentration; dnSNARE, dominant negative SNARE; Sb2, synaptobrevin 2; SNARE, soluble N-ethyl maleimide-sensitive fusion protein attachment protein receptor; TCA, tricarboxylic acid; TIRF, total internal reflection fluorescence; VNUT, vesicular nucleotide transporter.

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released from astrocytes via Ca^{2+}-dependent regulated exocytosis (Parpura, 1994). The later mechanism led to the discovery of gliotransmission-based modulation of synaptic transmission (Araque et al., 1998). These results, and many that followed, established the concept that a synapse consists of at least three elements, first termed the ''synaptic triad'' (Kettenmann et al., 1996), and later the ''tripartite synapse'' (Araque et al., 1999). Thus, in addition to the classical pre- and post-synaptic elements, astrocytes provide the third synaptic element that contributes an additional functional level of complexity in synaptic physiology. This concept has evolved further into the multi-partite synapse which in addition includes the extracellular matrix and microglial processes (Dityatev and Rusakov, 2011; Verkhratsky and Nedergaard, 2014). Since astrocytes can detect synaptic activity and signal back to neuronal networks with the release of chemical transmitters mainly using the vesicular exocytosis as an underlying mechanism governing this secretion, in this review we overview some aspects of vesicular architecture and dynamics of these organelles in astroglial cells.

MECHANISMS OF GLIOTRANSMITTER RELEASE FROM ASTROCYTES: EMINENCE OF REGULATED EXOCYTOSIS

Several mechanisms of gliotransmitter release appear to coexist in a single astrocyte (Parpura and Zorec, 2010; Parpura and Verkhratsky, 2012). In addition to (i) the vesicle-based mechanisms, astroglial cells can release chemical messengers through: (ii) plasmalemmal channels like, for example, opening of anion channels, induced by cell swelling (Pasantes Morales and Schousboe, 1988; Kimelberg et al., 1990) or by an increase in cytosolic calcium (Woo et al., 2012); through (iii) unpaired connexons or pannexons, generally known as ''hemichannels'', on the cell surface (Cotrina et al., 1998; Ye et al., 2003; Iglesias et al., 2009); through (iv) ionotropic purinergic $P2X_7$ receptors (Duan, 2003); through (v) plasma membrane excitatory amino acid transporters, by means of the reverse operational mode (Szatkowski et al., 1990); and (vi) by exchange via the cystine-glutamate antiporter (Warr et al., 1999). Here we shall narrate several key concepts, supporting the notion that astroglial cells, similar to majority of eukaryotic cells, utilize vesicle-laden membrane storage organelles to mediate secretion.

Vesicle-based chemical messenger release is mediated by the process of exocytosis, which involves the fusion between the vesicular and plasma membranes. This universal process, an evolutionary invention of eukaryotic cells, emerged in an ancestral cell by endosymbiosis. When ancestral eukaryotic cells increased in volume and complexity, subcellular organelles (with specialized functions), including mitochondria, lysosomes, Golgi organelles and secretory vesicles emerged. Increased cell volume also affected intracellular diffusion in such a way that concentrating signaling molecules in the cytosol became energetically ''expensive'', hence it became more ''economical'' to store signaling molecules in smaller volume organelles at ''low energy cost'' (Guček et al., 2012) see also (Attwell and Laughlin, 2001).

Vesicular secretion is dynamically and spatiotemporally regulated. By having secretory vesicles clustered close to the plasma membrane, for example, in an active zone – a special morphological feature of the presynaptic terminal – the delay of signaling to the neighboring postsynaptic membrane is minimized being as short as 100 μs (Sabatini and Regehr, 1999). The vesicle-based mechanisms of chemical messenger release, however, can exhibit much longer delays, for example, by regulating kinetics of the delivery of the secretory vesicle to the subcellular membrane signaling sites. In addition, vesicular discharge can be not only decelerated but also its content can be reduced by regulating kinetics of the vesicle fusion pore formation and/or fusion-pore diameter. Arguably, it seems that in astrocytes mechanisms prolonging the time between the arrival of the stimulus and the release of transmitters have been naturally selected, since the maximal speed of regulated exocytosis in astrocytes appears much slower in comparison to that in neurons (Guček et al., 2012; Neher, 2012). At present, astrocytes appear as the slowest secretors of all the excitable mammalian cells investigated thus far. However, it is this sluggish exocytotic secretagogue-secretion coupling mechanism that makes astrocytes an excellent model to study this process.

REGULATED EXOCYTOSIS IN ASTROCYTES IS SLOW

The properties of vesicle-based mechanisms of gliotransmission can be studied at the cellular level by monitoring changes in the plasma membrane area, since the fusion of the vesicle with the plasmalemma contributes to changes in the surface membrane area. This can be monitored by measuring membrane capacitance (C_m), which is linearly related to the membrane area (Neher and Marty, 1982). This technique was used in cultured astrocytes (Kreft et al., 2004), to test the hypothesis that an increase in cytosolic calcium concentration ($[Ca^{2+}]_i$), following photolysis of caged Ca^{2+} (Neher and Zucker, 1993), elicits an increase in the whole-cell C_m. Half-maximal increase in C_m of these astrocytes was attained at around 27 μM of $[Ca^{2+}]_i$, which is similar to the Ca^{2+}-dependency of the regulated exocytosis in various types of neurons, recorded by a similar technique (Heidelberger et al., 1994; Bollmann et al., 2000; Kreft et al., 2003). In contrast to neurons, however, a rather small, within 100 nM, increase in $[Ca^{2+}]_i$ from the resting level was sufficient to induce glutamate release from astrocytes, as detected by glutamatergic effects on nearby neurons, used as sniffer cells (Parpura and Haydon, 2000). A similar high-affinity calcium-sensing mechanism for vesicular release was reported in pituitary endocrine cells (Kreft et al., 2003).

Moreover, the kinetics of C_m increase revealed that in astrocytes the rate of exocytosis is at least two orders of magnitude slower in comparison to the kinetics of regulated exocytosis recorded by a similar technique in neurons (Kreft et al., 2004). The relatively slow