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Astrocytes Use a Novel Transporter to Fill Gliotransmitter Vesicles with D-Serine: Evidence for Vesicular Synergy

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Review of Martineau et al.

Glia cells play an active role in regulating and maintaining basic brain function. Through the release of various signaling molecules, known as gliotransmitters, they can influence the activity of neighboring neurons and other glia. D-serine and glutamate are two well studied putative gliotransmitters that have been shown to play important roles in neuronal activation and synaptic plasticity. However, the mechanisms mediating their storage and release remain unclear. In particular, whether the delivery of these gliotransmitters is mediated by Ca²⁺-regulated exocytosis or through nonexocytotic release from cytosolic pools via transporters or channels is debated (Santello et al., 2012). Furthermore, it is unknown whether exocytotic release occurs mainly through synaptic-like vesicles, lysosomes, or large vesicles (Kang et al., 2013).

There is mounting evidence suggesting that astrocytes can release gliotransmitters via synaptic-like vesicle exocytosis. First, astrocytes have the necessary secretory machinery for exocytosis, including the soluble N-ethyl maleimide-sensitive fusion protein receptor (SNARE) complex (Malar-

key and Parpura, 2008). Second, small synaptic-like vesicles containing glutamate and D-serine have been identified in glia (Bezzi et al., 2004, Bergersen et al., 2012). Third, there is evidence that both D-serine and L-glutamate release are regulated by calcium and rely on vesicle-associated membrane protein (VAMP)-dependent exocytosis (Santello et al., 2012).

However, reports exist wherein abolishing or enhancing Ca²⁺ transients in astrocytes of mice has no obvious consequences, putting into question the physiological significance of Ca²⁺-regulated gliotransmission (Agulhon et al., 2008). Furthermore, previous studies have not directly shown that D-serine is released via exocytosis and have left open the possibility that experimental manipulations inhibited the insertion of transporters or ion channels. Finally, even if gliotransmission is mediated by exocytosis, it has been questioned whether this would result in sufficient extracellular gliotransmitter concentration to be the principal pathway of release (Takano et al., 2005).

In an attempt to elucidate the nature of the gliotransmitter release process and the components involved, Martineau et al. (2013) immunopurified Sb2 (VAMP2)-positive organelles from cultured cortical astrocytes of newborn rats (Martineau et al., 2013). Martineau and coworkers (2013) provide support for previous findings that glia contain small gliotransmitter vesicles (GVs), sized ~40 nm (Bergersen et al., 2012). These vesicles appeared to be associated with vesicular gluta-

mate transporter 2 (vGlut2), non-neuronal isoform of the VAMP family cellubrevin/VAMP3, and synaptic vesicle protein 2 (SV2). They also showed, using capillary electrophoresis, that these vesicles contained D-serine and L-glutamate. Interestingly, the presence of large amounts of glycine was detected as well, but this was not discussed. GABA, another putative gliotransmitter, was not identified, suggesting that GABA is likely to be released from glia by a nonvesicular type mechanism.

At the same time, when Martineau et al. (2013) isolated synaptic vesicles (SVs) from neurons, they found that SVs contain L-glutamate, glycine, and GABA, but not D-serine. This finding is consistent with several other recent results (Rosenberg et al. 2010, 2013). Indeed, in the same issue of *The Journal of Neuroscience*, Rosenberg et al. (2013) provided evidence that D-serine release from neurons is mediated via the amino acid transporter Asc-1 as opposed to synaptic vesicular release.

In addition to identifying D-serine and L-glutamate within GV, Martineau et al. (2013) identified the existence of a vesicular transporter specific for D-serine uptake into GV. They found that uptake is driven by a proton electrochemical potential generated by V-ATPase, similar to what has been described for glutamate uptake in SVs. Furthermore, they show that chloride provides the necessary charge balance for protons during D-serine, as well as for L-glutamate, uptake. D-serine and glutamate uptake were both stimu-

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lated at low chloride concentrations (4 mM) and inhibited at higher concentrations (100 mM).

Interestingly, Martineau et al. (2013) found that D-serine application modulates the uptake of L-glutamate and vice versa, suggesting that these two amino acids may be released from the same vesicles. Notably, D-serine had no effect on L-glutamate uptake into SVs, which excluded the possibility that D-serine was directly acting on vGlut. Thus, Martineau et al. (2013) provide the first example of vesicular synergy in gliotransmitter vesicles.

Surprisingly L-serine, which was not detected within GVs, also seemed to induce the uptake of D-serine into GVs, as measured by a vesicular acidification assay. However, if the astrocytes were preloaded with D-serine, L-serine had no effect on acidification. This finding led the authors to investigate whether serine racemase (SR), the enzyme that converts L-serine to D-serine, was present within GVs. Indeed, significant colocalization of SR and Sb2 was identified by confocal microscopy. This finding is interesting because it is currently debated whether D-serine is synthesized primarily in astrocytes or neurons (Wolosker, 2011) and these data support the possibility that astrocytes are locally synthesizing D-serine at the level of GVs.

Overall, the observations by Martineau et al. (2013) help to solidify the hypothesis that D-serine and L-glutamate release from glia depends, at least in part, on VAMP proteins. The authors do not demonstrate this directly, but it is assumed because Sb2 (VAMP2) and cellubrevin (VAMP3) are attached to the vesicles. Moreover, they show that D-serine and L-glutamate mutually aid each other's loading through vesicular synergy and suggest that they may be stored together in the same vesicles. Martineau et al. (2013) also suggest the existence of a novel D-serine transporter and discover that some D-serine synthesis is occurring locally at gliotransmitter vesicles.

Although Martineau et al. (2013) provide evidence that D-serine and L-glutamate are loaded into and stored in small vesicles, this does not exclude the possibility of release through other mechanisms. Several

additional astrocytic release pathways have been identified, including volume-regulated anion channels (VRACs), glutamate exchange via the cystine-glutamate antiporter, release through ionotropic purinergic receptors or hemichannels, and exocytotic release through lysosomes or other vesicles. For example, activation of VRACs has been shown to result in a significant amount of D-serine release from astrocytes (Rosenberg et al., 2010). Rosenberg et al. (2010) also found that blocking vesicular filling using bafilomycin and concanamycin, which are inhibitors of vacuolar ATPase, did not affect D-serine release. This does not necessarily contradict the findings of Martineau et al. (2013), which found that bafilomycin blocked uptake of D-serine into GVs, because Martineau et al. (2013) did not look at the release properties of GVs. The contribution of release by small GVs could constitute only a small fraction of total glial D-serine release, as has been pointed out previously (Takano et al., 2005). It is also of interest that a recent study has reported storage and Ca^{2+} -dependent release of D-serine from hippocampal astrocytes by large vesicles with a diameter of up to 3 μm (Kang et al., 2013). According to this study, large vesicles are formed by the fusion of small vesicles, the smaller vesicles on their own being inefficient in exocytosis. However, the formation of large vesicles was mainly observed during sustained high astrocytic $[\text{Ca}^{2+}]$ or after weak mechanical stimulation, which are situations that are more likely to occur in pathological conditions.

These studies establish that small synaptic-like glial vesicles exist and contain gliotransmitters; however, additional studies are needed to further investigate the relative contribution of small vesicular gliotransmitter release versus other known release pathways. In view of the compartmental organization of astrocytes, it is tempting to speculate that small vesicles might serve as a local signaling mechanism on single synapses, whereas channels, transporters, or large vesicles mediate more voluminous release and thus affect many synapses simultaneously. It is also important to consider that gliotransmitter release mechanisms may be influenced by specific

conditions or stimuli (e.g., normal vs pathological), age, and brain area.

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