Dense Core Vesicle Release: Controlling the Where as Well as the When

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ABSTRACT Ca^{2+}/calmodulin-dependent Kinase II (CaMKII) is a calcium-regulated serine threonine kinase whose functions include regulation of synaptic activity (Coultrap and Bayer 2012). A postsynaptic role for CaMKII in triggering long-lasting changes in synaptic activity at some synapses has been established, although the relevant downstream targets remain to be defined (Nicoll and Roche 2013). A presynaptic role for CaMKII in regulating synaptic activity is less clear with evidence for CaMKII either increasing or decreasing release of neurotransmitter from synaptic vesicles (SVs) (Wang 2008). In this issue Hoover *et al.* (2014) further expand upon the role of CaMKII in presynaptic cells by demonstrating a role in regulating another form of neuronal signaling, that of dense core vesicles (DCVs), whose contents can include neuropeptides and insulin-related peptides, as well as other neuromodulators such as serotonin and dopamine (Michael *et al.* 2006). Intriguingly, Hoover *et al.* (2014) demonstrate that active CaMKII is required cell autonomously to prevent premature release of DCVs after they bud from the Golgi in the soma and before they are trafficked to their release sites in the axon. This role of CaMKII requires it to have kinase activity as well as an activating calcium signal released from internal ER stores via the ryanodine receptor. Not only does this represent a novel function for CaMKII but also it offers new insights into how DCVs are regulated. Compared to SVs we know much less about how DCVs are trafficked, docked, and primed for release. This is despite the fact that neuropeptides are major regulators of human brain function, including mood, anxiety, and social interactions (Garrison *et al.* 2012; Kormos and Gaszner 2013; Walker and Mcglone 2013). This is supported by studies showing mutations in genes for DCV regulators or cargoes are associated with human mental disorders (Sadakata and Furuichi 2009; Alldredge 2010; Quinn 2013; Quinn *et al.* 2013). We lack even a basic understanding of DCV function, such as, are there defined DCV docking sites and, if so, how are DCVs delivered to these release sites? These results from Hoover *et al.* (2014) promise to be a starting point in answering some of these questions.

FOR this study Hoover *et al.* (2014) visualized DCVs in *Caenorhabditis elegans*, using both GFP fusions and antibody markers. A variety of dense core vesicle (DCV)-specific GFP-tagged proteins were used, including transmembrane proteins, processed neuropeptides, and nonprocessed peptide cargoes. The authors examined DCV distribution in a class of motor neurons that have their dendrites and soma on the ventral side of the animal and release acetylcholine only on the dorsal side to trigger dorsal muscle contraction. This has the advantage that the dendrite, soma, and axon can be easily distinguished (Sieburth *et al.* 2005). Mutations in the single *C. elegans* Ca^{2+}/calmodulin-dependent Kinase II (CaMKII) gene, *unc-43*, caused a loss of all DCV markers in

the axons and this was confirmed using electron microscopy to directly visualize the DCVs. Conversely synaptic vesicles (SVs) were much less affected by mutations in CaMKII. Equally reduced were the numbers of DCVs in the commissures, presumably en route to the axons. Thus, in CaMKII mutants there was a defect in the ability of DCVs to exit the soma. And yet DCVs did not accumulate in the soma or anywhere else in the cell. So where had the DCVs and their cargo gone?

One possibility was that the DCVs were being inappropriately trafficked to lysosomes and thus destroyed. However, although the DCVs themselves had disappeared, their internal cargoes could be seen to be accumulating in another set of cells, the coelomocytes. The coelomocytes are six cells spaced in pairs along the length of *C. elegans*. They endocytose the extracellular fluid of the animal into which peptides contained within DCVs are released (Sieburth *et al.* 2007). Hence, GFP-tagged markers used in this study, once

released, can be observed accumulating within the coelomocytes. The purpose of these cells remains unclear, but they provide a very useful tool for researchers to observe peptide release from cells. In the CaMKII mutants Hoover *et al.* (2014) observed an increased level of DCV release compared to that in wild-type animals. The conclusion was that the reason the DCVs were missing from the motor neurons in the CaMKII mutant was because they had fused with the plasma membrane and released their cargoes at a much higher rate than in wild-type animals. Interestingly, even transmembrane DCV markers were decreased. This suggests a very rapid endocytosis and destruction of DCV transmembrane proteins from the plasma membrane after DCV fusion.

The question then became, why was DCV release increased in the CaMKII mutants? As very few DCVs were exiting the soma, the release must have been happening within the soma. The DCVs are normally docked and then held at the membrane and released only in response to a rise in intracellular calcium. In the CaMKII mutants could the DCVs have lost their identity and become vesicles in a constitutive release pathway, *i.e.*, calcium independent? To answer this the authors tested for the requirement of the calcium activator of protein secretion (CAPS), which is specifically required for DCV release (Speese *et al.* 2007). In the CaMKII mutants the CAPS mutant blocked the elevated release of DCV contents and substantially increased the total number of DCVs present in the axon; in contrast, release of cargoes through the normal constitutive pathway was unaffected by the CAPS mutation. This allowed the authors to make two important conclusions. First, in the CaMKII mutant animals the DCVs retained their identity and were not being treated as vesicles that were part of a constitutive release pathway. Second, the CaMKII mutation was not preventing the trafficking of DCVs to the axon. As long as premature release was blocked, then the DCVs trafficked to where they should have been. This lack of a trafficking defect is important as studies in *Drosophila* had implicated both CaMKII and the ryanodine receptor as regulators of DCV trafficking and there was no indication of a premature release defect (Wong *et al.* 2008). However, these *Drosophila* studies did not test genetic mutations but instead depended on the CaMKII inhibitor KN-93, which has been shown to alter the activity of kinases and ion channels in addition to its effects on CaMKII, indicating that these results need to be treated with caution (Ledoux *et al.* 1999).

What then is the role for CaMKII in inhibiting DCV release in the soma? One possibility was that the soma had a higher resting-state level of calcium. The DCVs once budded from the Golgi were likely to possess all the proteins required for calcium-dependent release. Thus, within the soma the DCVs could have been exposed to a high enough concentration of calcium to trigger DCV release and this must be prevented. What better mechanism to ensure this than a calcium-regulated kinase, which, when activated, inhibits release? This would also explain why only DCVs and not SVs were affected by CaMKII mutations as SV proteins are trafficked via synaptic vesicle precursors that are not converted to release competent

synaptic vesicles until they reach release sites at the axon (Bonanomi *et al.* 2006). However, arguing against this model is that mutations in the ryanodine receptor also elevated DCV release in the soma albeit to a lesser extent than the CaMKII mutants. Were CaMKII acting to prevent premature DCV release due to high calcium levels in the soma, then mutations in the ryanodine receptor would have been expected to reduce calcium levels and thus decrease premature DCV release, not increase it. Hoover *et al.* (2014) suggest that perhaps the soma is a site for DCV storage ready for trafficking to the axon in response to changes in activity or neuromodulatory signals. Alternatively, perhaps a switch from DCV release from the axon to release from the soma is a regulated process. Under certain conditions there could be an advantage for the significant increase in the rate of DCV release observed when CaMKII activity is reduced, possibly coordinated with a transcriptional switch that alters the neuropeptides and/or other cargoes contained within the DCVs. It is also possible that CaMKII activity is regulated within only the axons, thus altering the dynamics of DCV release at axons. Perhaps axonal DCV release is a balance between positive signals, such as diacylglycerol (Zhou *et al.* 2007), and negative signals such as CaMKII activity and these could be modulated to control levels of DCV release. Calcium's ability to inhibit and promote DCV release is likely to make it complicated to understand the regulation of DCV release.

Another important question is, how is the inhibitory signal lost? Hoover *et al.* (2014) observed that a gain-of-function CaMKII mutation resulted in an accumulation of DCV markers in axons, suggesting a defect in DCV release in the axon. This suggests that activated CaMKII remained associated with the DCVs in the axon and was capable of inhibiting DCV release even at its correct release sites. For wild-type animals perhaps as DCVs exited the soma calcium levels became lower, wild-type CaMKII activity ceased, and constitutively active phosphatases removed any inhibitory phosphorylations of DCV release. Of course, immediately prior to DCV release there must be a rise in calcium and yet this cannot activate the CaMKII inhibitory signal, or, if it does, then the exocytosis machinery for DCV release at the axon can bypass it. Perhaps wild-type CaMKII is no longer associated with DCVs in the axon, unlike gain-of-function CaMKII mutants. Critical to answering this question will be to determine what is (are) the target(s) of CaMKII that inhibit(s) DCV release.

In *C. elegans* CaMKII mutations cause defects in a number of behaviors, including locomotion, egg laying, and defecation (Reiner *et al.* 1999). In addition, CAMKII mutations alter levels of serotonin synthesis, aging, neuronal asymmetries, and transcription (Sagasti *et al.* 2001; Suo *et al.* 2006; Tao *et al.* 2013; Qin *et al.* 2013). Most, if not all of these behaviors can be regulated by neuropeptides (de Bono and Bargmann 1998; Nelson *et al.* 1998; Keating *et al.* 2003; Hu *et al.* 2011; Wang *et al.* 2013); however, it remains unclear whether all, some, or none of the phenotypes associated with mutations in CaMKII depend on its role in control of DCV release. Suppressors of the reduced locomotion and egg-laying phenotypes of gain-of-

function CaMKII mutants include two genes, *goa-1* and *dgk-1* (Robatzek *et al.* 2001), mutations in which cause increased levels of DCV release (Ch'ng *et al.* 2008). However, these mutations also increase neurotransmitter release from SVs (Perez-Mansilla and Nurrish 2009). Thus further work remains to determine whether the premature release of DCVs in CaMKII mutants constitutes a major reason for the observed CaMKII mutant phenotypes.

Finally, is this role for CaMKII specific to *C. elegans* or could it be conserved in other animals such as humans? At the post-synapse CaMKII regulates AMPA receptor trafficking in both mammals and *C. elegans*, demonstrating that at least some neuronal functions of CaMKII are conserved (Kaplan and Rongo 1999). In addition, the *C. elegans* CaMKII has been reported to alter neurotransmitter release via phosphorylation of a member of the Slo family of K+ channels (Liu *et al.* 2007) and there are suggestions that the orthologous channel in mammalian neurons can also be regulated by CaMKII (Wang 2008). Hoover *et al.* (2014) demonstrate the first example of a role of CaMKII in preventing premature DCV release and this may be unique to *C. elegans*. However, inappropriate release of DCVs before they reach their correct release sites would be a major problem in any species and I suspect this mechanism will prove to be a common one. This also suggests that the locations of DCV release are important and that there is some form of protein complex to assemble and maintain DCV release sites. Does the role of CaMKII in regulation of DCV release affect its role as a regulator of long-term changes in synaptic strength? It is too early to tell but it is an intriguing thought.

Acknowledgments

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