Distinct pools of synaptic vesicles are released by different calcium channels

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Abstract

Mueller et al. [1] uncover distinct roles for Ca_V1 and Ca_V2 channels in neurotransmitter release at the *C. elegans* neuromuscular junction. Although nanodomain coupling occurs via clustered Ca_V2 channels, evidence is also presented that release of a separate vesicular pool is mediated by more peripheral, dispersed Ca_V1 channels, requiring obligatory coupling with RYR to amplify the Ca^{2+} signal.

Introduction

Within the central and peripheral nervous systems, there are presynaptic terminals with many different functions, supported by varying architectures and vesicle density, and containing differing presynaptic calcium channel compositions. For example, neuromuscular junctions (nmj), and the calyx of Held synapse in the auditory pathway represent large terminals which follow faithfully and rapidly the arrival of an action potential, to make sure muscles contract and sound is heard accurately [for review see 2]. In contrast, many central synapses are much more plastic and can be fine-tuned in numerous ways [3].

Many previous studies have dissected how presynaptic voltage-gated calcium channels mediate vesicular release, in terms of the number and subtypes of channels and where they are anchored relative to the vesicles [for review see 2]. In the mammalian central nervous system, Ca_V2 channels are closely coupled to vesicular release, and Ca^{2+} entry mediates action potential-induced release. Further, during development, coupling becomes more associated with a nanodomain of very closely-linked Ca_V channels. Some previous studies have indicated $Ca_V2.1$ channels are more tightly coupled with the vesicle release sites than $Ca_V2.2$ [4], and that other channels (particularly $Ca_V2.3$) can be involved in asynchronous release [5].

Presynaptic roles for Ca_V1 (L-type) channels are well known for certain synapses, including auditory hair cells and retinal ribbon synapses [for review see 2]. In other tissues, L-type channels are preferentially coupled to intracellular ryanodine receptors (RYR), either directly (in skeletal muscle) or indirectly via Ca^{2+}-induced Ca^{2+} release (CICR) in cardiac muscle. There are only a few previous studies investigating the roles of presynaptic endoplasmic reticulum (ER), with respect to release of their intracellular Ca^{2+} stores. Indeed, De Juan-Sanz et al [6] found neuronal activity results in net Ca^{2+} uptake into presynaptic ER. Several earlier studies showed a role for presynaptic L-type channels; for example, in presynaptic long-term potentiation, in which L-type channels may act as a primary Ca^{2+} source, to secondarily activate presynaptic RYR [7]. Furthermore, Narita et al [8] showed that RYR are close to Ca_V channels in the frog nmj and amplify synaptic release and plasticity via CICR.

Distinct coupling of Ca_V1 and Ca_V2 channels to RYR in *C. elegans* nmj

The present study [1] examines presynaptic function in the nematode *C. elegans* nmj, which contains two main calcium channels Ca_V1 (egl-19) and Ca_V2 (unc2). Vesicle fusion rate at these synapses is graded, dependent on presynaptic depolarization. The study examines mini-epsc (mepsc) frequency, which reflects spontaneous tonic vesicular release of the transmitters, acetylcholine and

GABA. In some studies they isolate cholinergic mepscs, for better interpretation of their quantal data. It has previously been described that Ca_V1 and Ca_V2channels are both involved in tonic release from these synapses [9]. The present study has uncovered a surprising obligatory role of presynaptic RYR (unc 68) in Ca_V1 function, and also that spatially separate vesicular pools are involved [1].

C. elegans is particularly tractable to genetic studies to enable the knock-out or knock-down of different components of the pathway, where necessary only in the nervous system. This is essential as RYR and L-type channels are essential for muscle function. The worms were initially studied with behavioral testing, to examine their phenotype. To back up these genetic manipulations, the authors also employed a number of pharmacological tools, particularly the dihydropyridine nemadipine to block L-type channels, and dantrolene to block RYR. They also use electron microscopy to examine the distribution of vesicles, and numbers of docked vesicles, relative to the central dense projection. In addition, they employed a super-resolution fluorescence microscopy technique, together with tagged versions of the channel proteins.

There are several key findings of this extensive study. Firstly, Ca_V2 channels were found to be highly clustered, colocalized with the priming protein UNC-13L, and Ca^{2+} entry through these channels resulted in fusion of vesicles docked within about 33 nm of the dense projection, representing classical nanodomain vesicular release. About 100 Ca_V2 channels were estimated to be present per dense projection-associated cluster, and with high activity, multivesicular release may occur, and it can be amplified by CICR from RYR to involve a larger release domain (Fig. 1).

Secondly, and by contrast, Ca_V1 channels are much more scattered on the membrane of the presynaptic terminal, where they are colocalized with UNC-13S. RYR are found to be closely apposed to these channels, and fusion of a distinct vesicle population mediated by Ca^{2+} entry through Ca_V1 channels is completely dependent on the release of Ca^{2+} from internal stores via RYR (Fig. 1). The study leaves open the possibility that, as well as CICR, there may also be direct coupling of presynaptic Ca_V1 channels to RYR. Furthermore, Ca_V1 channels are slowly inactivating and could also be coupled to Ca^{2+}-activated K^+ channels and hyperpolarise synapses to mediate inhibitory modulation of release over time.

The complex interaction of presynaptic Ca_V1 and Ca_V2 channels with RYR that is uncovered in this study [1] will affect the speed and quantal content of vesicular release, dependent on presynaptic depolarization levels. These results beg many further questions for future studies, such as whether the distinct synaptic vesicle pools, which are released by the different Ca_V channels, might have different properties in terms of content, associated proteins and recycling kinetics. Furthermore, the important role of presynaptic ER in vesicular release, which is further established here [1], may also play a role in disease processes [10].

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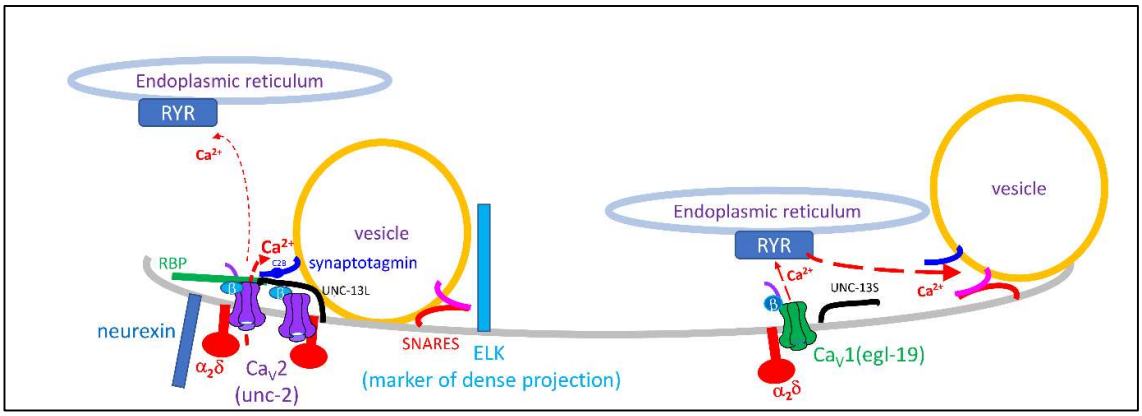
Figure 1



Diagrammatic distribution of Ca_V1 and Ca_V2 channels relative to synaptic vesicle release sites in *C. elegans* nmj, in association with some of the proteins involved in presynaptic release [1]. ELKS is a scaffolding protein that is used in the study as a marker for the dense projection, which is similar to the T-bar in Drosophila nmj or the ribbon in ribbon synapses. Adapted from Fig 4a in [2].

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