THE ROLE OF COMPLEXIN IN NEUROTRANSMITTER RELEASE

Student Researcher: Joel Fernandez
Mentor: Jeremy Dittman, Weill Cornell Medical College, New York, NY

Neurons communicate using specialized connections called synapses by releasing synaptic vesicles (SVs) containing neurotransmitters. How this process is regulated is not completely understood, although some of the key molecules have been identified. Complexins are a family of protein found primarily in the nervous system of all animals. We investigated the role of complexin I (*cpx-1*) in the nematode *C. elegans*. We found that *cpx-1* null mutants paralyzed far quicker in aldicarb than wild-type animals. Furthermore, the null mutants displayed severe locomotory defects; the null mutants showed about a 70% decrease in body bends per 20 seconds. Introducing a complexin GFP fusion protein into GABA motor neurons alone did not rescue the mutants; these animals displayed the exact defects as the null mutants, suggesting that null defects are independent of the GABA motor neurons. However, putting the same complexin back into all neurons did rescue the null mutants; rescue animals paralyzed on aldicarb with a wild-type time course and showed about a 150% increase in body bends than the null mutant. Our data suggest not only that complexin plays a critical role in neurotransmitter release but also that complexin is an inhibitor of this process. Furthermore, our data also imply that including a C terminal GFP does not impair complexin function. Through further analysis of complexin, we hope to better understand the protein's function in regulating synaptic transmission.

INTRODUCTION

Chemical synapses play a critical role in the function of an animal's nervous system. They are the means through which neurons transfer information. The presynaptic terminal is pivotal in this exchange. It is responsible for the regulated release of vesicles containing neurotransmitters, the chemicals which relay messages across synapses. How this vital and rapid process is regulated is still a mystery, although many key players have been identified.

The basic fusion machinery is the SNARE complex, a complex made of three proteins: SNAP-25, synaptobrevin, and syntaxin. The complex alone is sufficient to drive membrane fusion.[1] However, it is insufficient to drive fast, synchronous release; other proteins are needed for this process.

One of these proteins is complexin. Complexins are a family of protein found primarily in the nervous system of all animals. Mammals contain four complexin proteins (*CplxI, CplxII, CplxIII, CplxIV*). Complexin is known to bind to the SNARE complex.[2] However, its function is unknown. There is evidence to suggest that complexin acts as a ''fusion clamp'' and thus inhibits exocytosis.[1] However, recent experiments in mice suggest that complexin in fact facilitates synaptic exocytosis.[3]

In order to test complexin *in vivo*, we used the nematode *Caenorhabditis elegans* as knocking out complexin does not kill this model organism. *C. elegans* has a simple nervous system made up of only 302 neurons. Furthermore, *C. elegans* has only two complexin genes (*cpx-1, cpx-2*), of which *cpx-1* is an ortholog of mammalian *CplxI* and *CplxII*. The worm's transparency is helpful in imaging fluorescently-tagged proteins *in vivo*. Furthermore, many synaptic mutants have already been identified (a complexin null mutant among them).

Using this organism, we tested whether complexin plays an inhibitory or facilitating role in neurotransmitter release. Furthermore, we wanted to know if a null mutant can be rescued by reintroducing complexin into the animal.

METHODS

To test the function of complexin in synaptic exocytosis, we utilized an available *C. elegans* complexin null mutant. The mutant allele contains a large deletion including much of the coding region. This mutation is recessive, as heterozygous animals appear wild-type.

In order to test synaptic activity in the mutant, we used the aldicarb assay. Aldicarb blocks the enzyme acetylcholinesterase, which is responsible for the breakdown of acetylcholine (ACh) in the synaptic cleft. Continued release of ACh causes prolonged excitation of the muscle, leading to paralysis. Thus, increases in ACh levels at the synapse increase sensitivity to aldicarb.

Aldicarb Assays. Approximately 20 wild-type, *cpx-1* null mutant, and transgenic rescue animals (per trial) were coded (in order to perform the assay blind) and transferred onto 1 mM aldicarb plates. Animals were scored for paralysis every 10 minutes for two hours, beginning 20 minutes after transferring the animals onto aldicarb plates.

Locomotion Assays. Ten animals per genotype were placed on agar plates containing no food and left at 23° C for an hour. Body bends were counted per

20 seconds to quantify locomotion rates. Five animals were scored from each plate and three plates were score per genotype. A body bend was counted if a point posterior to the pharyngeal bulb moved right or left relative to the animal's midline.[4] Sample size was recorded in plate averages.

RESULTS

We found that the complexin null mutant paralyzed substantially quicker than wild-type animals, suggesting that complexin inhibits vesicle fusion.

To ensure that hypersensitivity to aldicarb was indeed due to the loss of complexin rather than some other defects, we introduced a full length rescuing complexin cDNA into null mutant animals using a pan-neuronal promoter. The transgenic animals were restored to near wild-type aldicarb sensitivity, suggesting not only that the aldicarb phenotype of null mutants is complexin dependent, but also that expressing complexin in neurons alone is sufficient to rescue the animals.

We also conducted a behavioral assay testing rates of locomotion of the nematode. Locomotion assays provide another means of assessing the role of complexin in the worm nervous system. Loss of a critical protein in synaptic exocytosis would result in severe locomotion defect. We assayed the locomotion of *cpx-1* null mutants to determine the severity of nervous system impairment. Indeed, *cpx-1* null mutants displayed a 70% decrease in locomotion (per 20 s), while the transgenic rescue animal displayed a 150% increase (as compared to the null mutants). This finding supported our aldicarb results.

The aldicarb sensitivity of *cpx-1* null mutants can also be explained by decreased GABA release at the neuromuscular junction (NMJ). If complexin plays a facilitating role in the GABAergic NMJs removal of complexin decreases GABA release, accounting for the increased sensitivity to aldicarb and locomotion defects. To test whether this was the case, complexin was restored in GABAergic neurons and assayed for rescue of aldicarb sensitivity and locomotion. We found that there was no significant difference in aldicarb sensitivity or locomotion rates among the GABA-specific rescue animals and the *cpx-1* null mutants, therefore supporting the hypothesis that complexin acts in cholinergic neurons to inhibit transmitter release.

DISCUSSION

Our results suggest two major conclusions. First, complexin plays an inhibitory role at synapses in *C. elegans*. Second, *cpx-1* null mutants can be rescued using a *cpx-1* GFP fusion protein.

Our first conclusion is consistent with previous experiments in mice. Overexpressing complexin in mice hippocampal cells strongly inhibited exocytosis, thus supporting the "fusion clamp" hypothesis.[1,5] However, recent experiments describe reduced calcium (Ca^{2+})-triggered exocytosis in complexin-knockout mice hippocampal cells, suggesting that complexin facilitates exocytosis.[3]

Another possible role for complexin has been described, which could explain the seemingly contradictory findings described above. There is evidence to believe that complexin stabilizes the SNARE complex in a primed state to allow for Ca^{2+}-evoked exocytosis instead of asynchronous release. Additionally, studies have shown that the protein synaptotagmin 1, which has been described as the neuron's calcium sensor, competes with complexin to bind to the SNARE complex.[5] In this model, calcium-bound synaptotagmin displaces complexin and allows for vesicle fusion. Therefore, any defects in Ca^{2+}-evoked exocytosis is not due to a facilitory role for complexin, but rather a supportive role.

In the future, we will employ quantitative *in vivo* imaging of synaptic complexin-GFP to better understand its recruitment and retention during synaptic activity. Furthermore, we will image complexin in various synaptic mutants to determine how complexin interacts with other proteins. We will also delete various domains of the protein to test the function of each domain in behavioral and imaging assays.

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