Synaptotagmin I Functions as a Calcium Sensor to Synchronize Neurotransmitter Release

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Finding the mechanism of late neurotransmitter release

- Hypothesis: Ca++ influx into the presynaptic nerve triggers neurotransmitter release (1967)
- Release happens in milliseconds even though vesicle must go through translocation, docking and priming
- Reconstitution of the SNARE components is slow and Ca++ independent (1998)
- Few neuronal preparations allow the control of Ca++ stimulus sufficiently for quantitative analysis
- The advancement of caged signaling compounds (1997) and genetic studies drove most analysis of process
What properties might this molecule possess?
Synaptotagmins

- Synaptotagmin have well characterized Ca++ binding motifs (1990)
- Synaptotagmin binds the SNARE complex and phospholipids in a Ca++ dept. manner (mid 90’s)
- Ca++ dept. interaction with t-SNARE and SNAP-25
Drosophila Syt Mutants

- **AD4= NULL = no interactions**; deletion of transmembrane and cytoplasmic domains

- **AD1 = NO C2B = little assc. with SNAREs**; deletion of C2B domain reducing Ca++ dept. assc with SNAREs and oligomerization, phospholipid binding preserved

- **AD3 = NO C2B Ca++ binding = no oligomerization**; Y364N in C2B does not abolish SNARE or phospholipid binding

- **N13 = NULL = no interactions**; Deleted at 5’ of gene so no protein made
Figure 1: Characterization of Mutants

A

<table>
<thead>
<tr>
<th>syt +</th>
<th>syt AD3</th>
<th>syt AD1</th>
<th>syt AD4</th>
<th>syt N13</th>
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<td>Synaptotagmin I</td>
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<td>ROP</td>
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<td>Syntaxin</td>
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<td>syt AD4</td>
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C

anti-HRP  anti-Syt  Merge
Electrophysiological Analysis

- Whole cell patch clamp to embryonic muscle fibers

- Motor nerves positioned at a suction electrode at the site of their emergence from the CNS

- Quantal content = ln(number of stimuli/number of failures of synaptic current w/ l 6ms)

- Mhc null mutant backgrounds to inhibit contractions
Figure 2: Mutants Disrupt Distinct Functions of Synaptotagmin
Figure 3: Molecular Features Required for Suppression of Asynchronous release
Fig 4. Comparison of Synchronous Release Cooperatively

A

\begin{align*}
\text{Mean Quantal Content} & \quad \text{Ca}^{2+} \text{ (mM)} \\
\text{sy}t^+ & \\
\text{sy}t^{AD3} & \\
\text{sy}t^{AD1} &
\end{align*}

B

\begin{align*}
\text{Mean Quantal Content in Log} & \quad \text{Ca}^{2+} \text{ (mM) in Log} \\
\text{sy}t^+ & \\
\text{sy}t^{AD3} & \\
\text{sy}t^{AD1} &
\end{align*}
Figure 5: Synaptotagmin role in Vesicle Recycling

A

B

Summed peaks of synaptic currents, nA x 3 sec

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C

Sucrose

D

Number of events for 5 sec

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Fig 6: Synaptotagmin function as a Fusion Clamp
Conclusions

• AD4, no synchronous release is observed
• AD1, can trigger synchronous release of vesicles, but in the absence of interaction with the SNARE complex there is a low release probability
• AD3, when this interaction with the SNARE complex is made, Ca++ dept cooperativity of release is seen and there is a higher probability of release
• Syt+, Ca++ dept oligomerization of C2B domain maximizes release probability
• Drawn stepwise but actual sequence of events in vivo unknown, likely to be simultaneously
Conclusions

• There are two kinetically and mechanistically distinct phases of release: a fast component (5-10ms) by a low affinity Ca++ sensor and a second distinct asynchronous component by a high affinity Ca++ sensor (100-200ms)

• In the AD1 mutant these synchronous and asynchronous phases coexist so the protein has the properties necessary to trigger the fast phase but cannot fully suppress the slow phase

• BEST EVIDENCE: cooperativity of neurotransmitter release is abolished in AD1, indicating this is the key Ca++ sensor

• Propose that syt protein rapidly triggers opening and stabilization of the fusion pore while preventing early opening at low Ca++ concentrations by the another high affinity sensor

• Syt does not have a role in docking and endocytosis

• Syt is a suppressor of delayed release during sustained Ca++ elevation, this is possibly mediated through the high affinity Ca++ sensor