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A discrete form of secretion

Action potentials trigger the influx of Ca^{2+} ions through voltage-gated calcium channels, which in turn leads to Ca^{2+}-dependent secretion (CDS) of vesicles containing neurotransmitters or neuropeptides from axon terminals or somata. Previous studies in sensory neurons of the dorsal root ganglion (DRG neurons) cultured in the absence of Ca^{2+} have identified an alternative form of somatic vesicle secretion that is triggered by action potentials, known as Ca^{2+}-independent but voltage-dependent secretion (CiVDS). However, the mechanisms underlying CiVDS have not been clear. Now, Zhou and colleagues characterize the molecular mechanisms mediating CiVDS and show that it results in the release of ATP and neuropeptide Y (NPY) from DRG neurons.

The authors reasoned that CiVDS probably requires a voltage sensor (likely to be found in voltage-gated channels), vesicle fusion machinery and a molecular means of linking the two. The authors tested the effects of various antagonists of voltage-gated channels on CiVDS — which can be detected as a 'jump' in membrane capacitance (C_m) elicited by depolarization in a Ca^{2+}-free solution — in freshly isolated mouse DRG neurons. CiVDS was strongly inhibited by an antagonist of N-type voltage-gated calcium channels (which contain the subunit $Ca_V2.2$). Moreover, short-hairpin RNA (shRNA)-mediated knock down of $Ca_V2.2$ in DRG neurons markedly reduced CiVDS, further supporting a role for these channels in CiVDS.

To determine which functions of the $Ca_V2.2$ channel are necessary for CiVDS, the authors introduced mutations in the voltage-sensing motif S4 or in the channel-pore region and expressed these mutant channels in DRG neurons that had been in culture for 3 days and had spontaneously reduced levels of endogenous $Ca_V2.2$ and impaired CiVDS compared with freshly isolated neurons. Whereas overexpression of wild-type or pore-mutant $Ca_V2.2$ rescued CiVDS in these cells, overexpression of S4-mutant $Ca_V2.2$ did not, implying that the voltage-sensing function of $Ca_V2.2$ is necessary for CiVDS.

Next, the authors hypothesized that vesicle release during CiVDS may be mediated by the SNAP receptor (SNARE) complex, as this mediates Ca^{2+}-dependent vesicle fusion in CDS. In line with this hypothesis, CiVDS was inhibited in DRG neurons by application of a combination of SNARE-protein-cleaving toxins. Furthermore, co-immunoprecipitation revealed that $Ca_V2.2$ in DRG neurons physically interacts with SNAP25, a SNARE protein. These results prompted the authors to ask whether $Ca_V2.2$ might bind directly to the SNARE complex to enable CiVDS. They truncated the synaptic protein interaction (synprint) site of $Ca_V2.2$; this synprint-truncated $Ca_V2.2$ did not bind SNAP25 and did not rescue CiVDS in DRG neurons that had spontaneously reduced CiVDS. Thus, the $Ca_V2.2$ synprint site links the voltage-sensing function of the channel with the SNARE machinery to enable CiVDS.

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What is the cargo released during CiVDS? A luciferase–luciferin assay demonstrated that ATP is released from depolarized neurons in Ca^{2+}-free solution. In addition, vesicles labelled with NPY–pHluorin were observed (using total internal reflection fluorescence (TIRF) microscopy) to be released upon depolarization in Ca^{2+}-free or Ca^{2+}-containing solution. Together, these data suggest that ATP and NPY may be released during CiVDS.

Overall, these results characterize the mechanisms underlying CiVDS. Specifically, they suggest that action potentials are detected by the S4 portion of the $Ca_V2.2$ channel, which in turn signals via its synprint region to the SNARE complex to trigger release of vesicles containing ATP and/or NPY. The authors note that CiVDS seems to dominate over CDS in DRG neurons at rest, and suggest that CiVDS may thus have a role in sensory homeostasis.

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