How do T-type calcium channels control low-threshold exocytosis?

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Low-voltage-activated T-type calcium channels act as a major pathway for calcium entry near the resting membrane potential in a wide range of neuronal cell types. Several reports have uncovered an unrecognized feature of T-type channels in the control of vesicular neurotransmitter and hormone release, a process so far thought to be mediated exclusively by high-voltage-activated calcium channels. However, the underlying molecular mechanisms linking T-type calcium channels to vesicular exocytosis have remained enigmatic. In a recent study, we have reported that Cav3.2 T-type channel forms a signaling complex with the neuronal Q-SNARE syntaxin-1A and SNAP-25. This interaction that relies on specific Ca v3.2 molecular determinants, not only modulates T-type channel activity, but was also found essential to support low-threshold exocytosis upon Ca 2+ channel expression in MPC 9/3L-AH chromaffin cells. Overall, we have indentified an unrecognized regulation pathway of T-type calcium channels by SNARE proteins, and proposed the first molecular mechanism by which T-type channels could mediate low-threshold exocytosis.

Depolarization-evoked synaptic transmission relies on the calcium (Ca 2+)-regulated release of quantal packets of neurotransmitters following fusion of synaptic vesicles with the presynaptic plasma membrane. 1 It is well established that neuronal voltage-gated Ca 2+ channels, by converting electrical signals into intracellular Ca 2+ concentration elevations, play a key role in triggering evoked neurotransmitter release. 2-4 Hence, Ca 2+ entry through high-voltage-activated (HVA) channels (N-, P/Q-, and in some extent and particular cell populations, L- and R-type) into presynaptic nerve terminals in response to action potentials supports a transient Ca 2+ microdomain essential for synaptic exocytosis. However, the observation that some neurons can release functionally significant amounts of neurotransmitter below the threshold of action potentials 6 questioned the possible involvement of another source of Ca 2+ ions, independent of HVA channels activation.

In contrast to HVA channels, low-voltage-activated (LVA) T-type Ca 2+ channels activate in response to subthreshold membrane depolarizations between -65 mV and -50 mV and thus represent an important source of Ca 2+ entry near the resting membrane potential. Hence, besides controlling important physiological processes by regulating neuronal excitability, pacemaker activity and post-inhibitory rebound burst firing, mounting evidences from various neuronal cell types suggest an efficient role of T-type channels in fast and low-threshold exocytosis. 7-10 Until now, however, the mechanism whereby these channels support exocytosis events at the molecular level remained a mystery. In a recent study, we provided compelling evidence of the existence of a Ca v3.2/syntaxin-1A molecular complex essential for T-type-dependent exocytosis. 11

In mammalian synapses, interaction of several members of the vesicle-docking / release machinery (including syntaxin-1A/1B and SNAP-25) onto a synprint (synaptic protein interaction site) domain located within the intracellular loop between domains II and III of the
Cav2.1 and Cav2.2 channels (Fig. 1A, top panel) ensures a close localization of the secretory vesicles near the Ca$^{2+}$ source. In turn, both synaptasin-1A/1B and SNAP-25 modulate calcium channel activity to fine tune Ca$^{2+}$ entry and synaptic strength. However, in contrast to HVA channels, all of the three T-type channel members (i.e. Cav3.1, Cav3.2 and Cav3.3) lack the consensus synprint site, making the molecular understanding of the involvement of these channels in the exocytosis process quite difficult. Our observation that Cav3.2 channels associate with syntaxin-1A in central neurons prompted us to investigate the possible existence of specific Cav3.2 channel molecular determinants other than the consensus HVA synprint domain. Using biochemical and cellular trafficking approaches, we demonstrated that syntaxin-1A, as well as SNAP-25, interact with the C-terminal domain of Cav3.2 channel (Fig. 1A, lower panel). Moreover, using patch-clamp recordings performed on tsA-201 cells expressing Cav3.2 channels, we demonstrated that co-expression of a syntaxin-1A in its "closed" conformational state (i.e. the conformation adopted by the syntaxin-1A in isolation or in interaction with Munc18) potently decreases Cav3.2 channel availability by shifting the voltage-dependence of inactivation toward more hyperpolarized membrane potentials, similarly to what was previously reported for N- and P/Q-type channels. Interestingly, this regulation was abolished upon co-expression of SNAP-25, and not observed with a constitutively "open" syntaxin-1A (i.e the conformation adopted upon its association with SNAP-25) (Fig. 1B). Given that syntaxin-1A undergoes a conformational switch from a "closed" to an "open" conformation during the vesicle release cycle, this suggests that syntaxin-1A may be able to dynamically regulate T-type channel availability during various stages of exocytosis. Interestingly, although T-type channels utilize distinct molecular determinants to interact with SNARE proteins (the C-terminal domain vs. the classical synprint of the II-III linker), they are subjected to a similar SNARE regulation. Does this observation question the molecular mechanism by which binding of syntaxin-1A produces changes in channel gating? Earlier reports have shown that reorganization of intramolecular interactions among the main intracellular loops of Ca$^{2+}$ channels critically influence channel inactivation. Mapping the intramolecular interactions of T-type channels along with the characterization of the minimal sequence engaged in the

Figure 1. SNARE proteins modulate high- and low-voltage-gated calcium channels via distinct molecular determinants. (A) Membrane topology of voltage-gated calcium channels highlighting the localization of the synprint site located within the intracellular linker between domains II and III of Cav2.1/Cav2.2 channels (top panel), and the "synprint like" domain of Cav3.x channels (bottom panel) located within the C-terminal domain of the channel. (B) Voltage-dependence of Cav3.x channel availability during the conformational switch of syntaxin-1A.
interaction with SNARE proteins will provide important structural information on how syntaxin-1A modulates channel gating. It is well known that direct interaction of SNARE proteins with Ca2+ channels is critical for depolarization-evoked neurotransmitter release. Hence, disruption of the Ca2+ channel-SNARE protein coupling by depletion of the synprint domain or by peptides derived from the synprint sequence, alters synaptic transmission. We revealed that similarly to HVA channels, T-type channels-mediated exocytosis relies on a channel-SNARE protein interaction. Indeed, membrane capacitance recordings performed on MPC 9/3L-HA chromaffin cells expressing Ca3.2 channels revealed robust voltage-dependent exocytosis which was totally prevented by co-expression of the Ca3.2 T-type C-terminal domain (i.e. the synaptic protein interaction site of Ca3.2). Ablation of Ca3.2-dependent exocytosis most likely results from the specific uncoupling of the channel with SNARE proteins and not from a side alteration of the exocytosis machinery by itself because no alteration was observed when exocytosis was induced by direct intracellular Ca2+ elevation. Hence, we showed that similarly to HVA channels, a physical coupling between SNARE proteins and T-type channels is critical for T-type-dependent exocytosis. Considering the relative small conductance of T-type channels and the restricted diffusion of Ca2+ due to the high Ca2+ buffering capacity of neuronal cells, it is conceivable that this interaction allows the close localization of the vesicle-docking/release machinery in close proximity to the Ca2+ source in order to efficiently sense Ca2+ elevation. However, we cannot exclude the possibility that interaction of T-type channels with SNARE proteins could form a macromolecular complex through which channel conformational changes following membrane depolarization would work as an on/off molecular switch of secretion by controlling the ultimate conformational change of the releasing complex as previously proposed for HVA channels. Although this concept still requires further investigation, the use on a non-conducting channel to investigate T-type-dependent secretion would definitely provide interesting information about the functional importance of T-type channel interaction with SNARE proteins.

Overall, we revealed an unrecognized regulation of low-voltage-activated T-type Ca2+ channels by SNARE proteins, and provide the first evidence for a molecular mechanism by which these channels could mediate low-threshold exocytosis. We revealed that, although T-type Ca2+ channels differ from HVA channels by their molecular constituents, they possess the same ability to functionally interact with SNARE proteins, highlighting a key evolutionary mechanism for specialized fast and spatially delimited exocytosis.

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