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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 $Ca_v3.2$ 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 $Ca_v3.2$ 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.

Keywords: calcium channel, T-type, $Ca_v3.2$ channel, SNARE, syntaxin-1A, SNAP-25, exocytosis, neuron, chromaffin cell, MPC cell

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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[5] 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* (*syn*aptic *pr*otein *int*eraction site) domain

located within the intracellular loop between domains II and III of the $Ca_v2.1$[12] and $Ca_v2.2$[13] channels (**Fig. 1A**, top panel) ensures a close localization of the secretory vesicles near the Ca^{2+} source. In turn, both synatxin-1A/1B and SNAP-25 modulate calcium channel activity[14,15] 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 $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$) lack the consensus *synprint* site, making the molecular understanding of the involvement of these channels in the exocytosis process quite difficult. Our observation that $Ca_v3.2$ channels associate with syntaxin-1A in central neurons prompted us to investigate the possible existence of specific $Ca_v3.2$ 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 $Ca_v3.2$ channel (**Fig. 1A**, lower panel). Moreover, using patch-clamp recordings performed on tsA-201 cells expressing $Ca_v3.2$ 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)[16,17] potently decreases $Ca_v3.2$ 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.[14,15,18-20] 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)[18] (**Fig. 1B**). Given that syntaxin-1A undergoes a conformational switch from a "*closed*" to an "*open*" conformation during the vesicle release cycle,[16,21,22] 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_v2 channels critically influence channel inactivation.[23-30] Mapping the

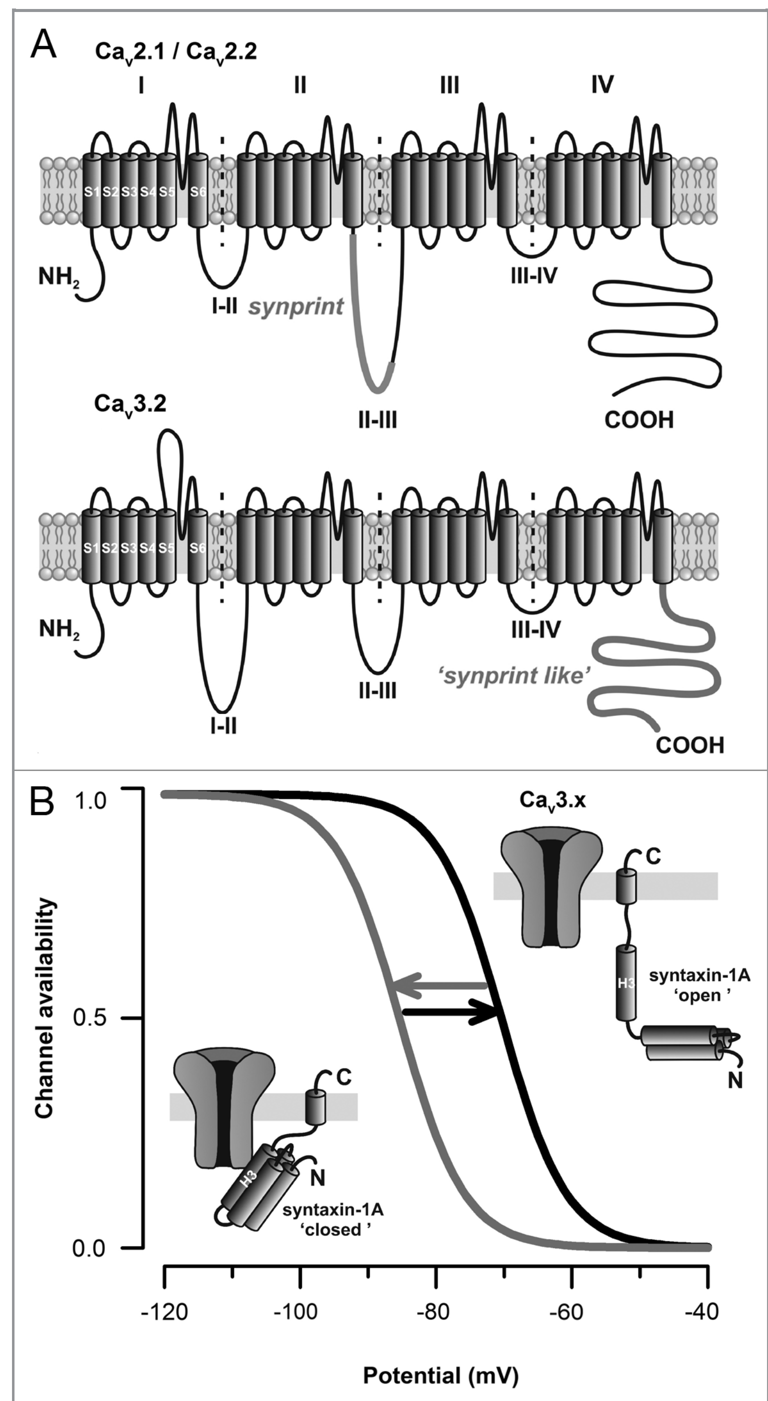

Figure 1. SNARE proteins modulate high- and low-voltage-gated calcium channels via distinct molecular determinants. (A) Membrane topology of voltage-gated calcium channels highlighting the localization of the *synprint* site located within the intracellular linker between domains II and III of $Ca_v2.1$/$Ca_v2.2$ channels (top panel), and the "*synprint* like" domain of $Ca_v3.x$ channels (bottom panel) located within the C-terminal domain of the channel. (B) Voltage-dependence of $Ca_v3.x$ channel availability during the conformational switch of syntaxin-1A.

intramolecular interactions of T-type channels along with the characterization of the minimal sequence engaged in the 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 $Ca_v2.1$ and $Ca_v2.2$ channels is critical for depolarization-evoked neurotransmitter release. Hence, disruption of the Ca^{2+} channel-SNARE proteins coupling by deletion of the *synprint* domain or by peptides derived from the *synprint* sequence, alters synaptic transmission.[31-34] 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 $Ca_v3.2$ channels revealed robust voltage-dependent exocytosis which was totally prevented by co-expression of the $Ca_v3.2$ C-terminal domain (i.e the synaptic protein interaction site of $Ca_v3.2$). Ablation of $Ca_v3.2$-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 Ca^{2+} 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[35] and the restricted diffusion of Ca^{2+} due to the high Ca^{2+} buffering capacity of neuronal cells,[36] it is conceivable that this interaction allows the close localization of the vesicle-docking / release machinery in close proximity to the Ca^{2+} source in order to efficiently sense Ca^{2+} 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.[37,38] Although this concept still requires further investigation, the use on a non-conducting channel to investigate T-type-dependent secretion would definitively 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 Ca^{2+} 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 Ca^{2+} 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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