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Masters or slaves? Vesicle release machinery and the regulation of presynaptic calcium channels

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Abstract

Calcium entry through presynaptic voltage-gated calcium channels is essential for neurotransmitter release. The two major types of presynaptic calcium channels contain a synaptic protein interaction site that physically interacts with synaptic vesicle release proteins. This is thought to tighten the coupling between the sources of calcium entry and the neurotransmitter release machinery. Conversely, the binding of synaptic proteins to presynaptic calcium channels regulates calcium channel activity. Hence, presynaptic calcium channels act not only as the masters of the synaptic release process, but also as key targets for feedback inhibition.

Keywords: Ca^{2+} channels; Syntaxin; SNARE proteins; Neurotransmission

1. Introduction

Calcium entry through presynaptic voltage-gated calcium channels (VGCCs) is a key step in neurotransmitter release [1]. Immunohistochemical studies have identified the N-type and P/Q-type Ca^{2+} channels as the dominant species present in the presynaptic C-terminal [2,3]. Not surprisingly, the two VGCC species responsible for the Ca^{2+} influx that supports presynaptic neurotransmitter release are specialized to interact with key proteins of the vesicle release machinery; specializations for channel/vesicle interactions are necessary to support the temporal and spatial requirements of releasing a vesicle within 200 μs after Ca^{2+} influx [4–7]. Several members of the vesicle release machinery, including syntaxin 1A and 1B, SNAP-25, VAMP2/synaptobrevin, synaptotagmin, and cysteine string protein (CSP) all bind to the synprint (*syn*aptic *pr*otein *int*eraction) motif of the II–III linker of N- and P/Q-type Ca^{2+} channels [8–18]. The notion that the vesicle release machinery modulates Ca^{2+} influx through VGCCs was initially proposed by three groups: Mastrogiacomo et al. in 1994 [10], working with CSP in *Drosphila melanogaster* and Bezprozvanny et al. 1995 [19] and Wiser et al. 1996 [20] who demonstrated in *Xenopus* oocytes that the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) proteins syntaxin 1A and SNAP-25 significantly reduced N-type Ca^{2+} channel availability and activity [10,19,20]. Recent work has uncovered a number of interactions between the presynaptic VGCCs and members of the vesicle release machinery that mediate Ca^{2+} influx through the channel; it appears that these proteins not only interact out of necessity for rapid vesicle release, but so that presynaptic proteins can regulate Ca^{2+} influx through the channels. This article will review the effects of various presynaptic proteins on Ca^{2+} influx, and provide an overview of how these proteins might work to efficiently regulate Ca^{2+} influx through presynaptic N- and P/Q-type Ca^{2+} channels in the absence or presence of docked vesicles. Although it is beyond the scope of this brief review, interested readers may refer to Atlas in 2001 [15] for a discussion of the interaction between vesicle release proteins and L-type Ca^{2+} channels [15]. Schematics of the interactions described in this review are presented in Figs. 1 and 2.

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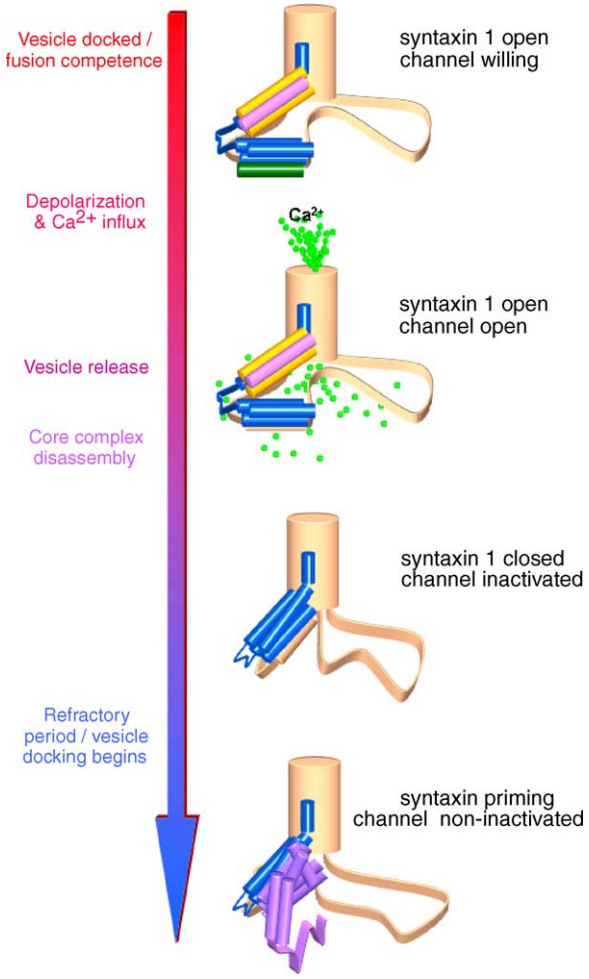

Fig. 1. A model for the regulation of calcium influx by vesicle-associated proteins during the vesicle release cycle. *Legend*: The presynaptic Ca^{2+} channel is shown as only the main pore-forming α_1 subunit with the II–III linker (light brown); syntaxin 1A (blue); SNAP-25 (golden); VAMP2 (pink); CSP (green); nSec-1 (purple). Top panel: syntaxin 1 is open and bound in the SNARE core complex with SNAP-25 and VAMP2. At this point, synaptotagmin (not shown) will also be associated, in addition to CSP, which is associated with the docked vesicle (not shown). At this stage, all potentials channel-inhibitory mechanisms are nullified by virtue of the various proteins being complexed, so potential Ca^{2+} is maximal. Also note that potential G-protein interaction with the channel/core complex is maximal at this stage. Second panel: following membrane depolarization, the channel opens, allowing the rapid Ca^{2+} influx which triggers vesicle release within 200 μs. Third panel: immediately after fusion, NSF activity (not shown) leads to SNARE core complex disassembly, which then allows syntaxin to adopt its most stable closed conformation, which stabilizes the inactivated state of the channel. Bottom panel: the solitary syntaxin 1 is a prime substrate for nSec-1 which binds following SNARE core complex disassembly. Experiments have shown that a channel expressed with syntaxin 1 and nSec-1 is not inactivated, suggesting that channels in this stage may allow Ca^{2+} influx although a vesicle is not docked [27]. nSec-1 binding to syntaxin 1 is followed by munc-13 binding (not shown), which initiates syntaxin 1 priming, prior to SNARE core complex assembly and vesicle docking.

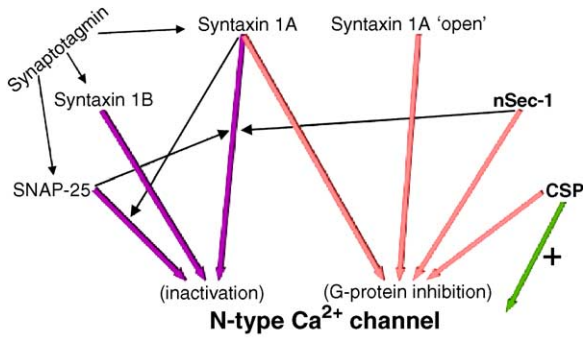

Fig. 2. Schematic representation of the interactions between the vesicle release proteins and the N-type calcium channel. *Legend*: Proteins of the vesicle release machinery mediate Ca^{2+} influx through the N-type channel via two distinct processes: (1) directly, by binding to the channel and stabilizing it's inactivated state, and/or (2) indirectly, but promoting the association of an inhibitory G-protein βγ subunit and the channel. Purple arrows indicate direct inactivation-promoting interactions with the channel. Peach arrows indicate indirect inhibition via Gβγ. Black arrows indicate interactions between presynaptic proteins. The green arrow indicates CSPs ability to promote increased channel activity. It should be noted that G-protein inhibition is not necessarily constant, but enhances channel inhibition by activated G-proteins.

2. Modulation of calcium channels by SNARE proteins

2.1. Syntaxin 1

Syntaxin 1A was originally identified in the presynaptic zones of neurons where it localized with synaptotagmin and the N-type Ca^{2+} channel [8,9]. Syntaxin 1 plays a central role in the process of exocytosis by contributing, with SNAP-25 and synaptobrevin/VAMP2, to the core complex which 'zippers' together and brings the vesicle and plasma membrane into close apposition [21,22]. Syntaxin 1A is anchored at the plasma membrane by a C-terminal transmembrane domain, and has been well studied for its interaction with the synprint site of the α_{1B} subunit of the N-type VGCC subunit [11,17,23,24]. By anchoring itself to the synprint motif, syntaxin 1A, along with associated Ca^{2+}-sensitive vesicle release machinery and the vesicle, is localized to within 50 nm of the source of Ca^{2+} [6,11,25], thus allowing for efficient vesicle release. Bezprozvanny et al. in 1995 [19] demonstrated that syntaxin 1A could, in fact, modulate both N-type and P/Q-type channels, shifting the steady-state inactivation by nearly −20 mV, which significantly reduced the numbers of available channels at resting potential [19]. Additional evidence supporting syntaxin 1-dependent channel inhibition comes from a number of subsequent studies in oocytes [20,26], HEK cells [16,27], synaptosomes [28], and chick ciliary ganglion calyces [29].

Another well-studied characteristic of the N-type Ca^{2+} channel is its susceptibility to G-protein inhibition. Subsequent to the initial discovery of this phenomenon [30,31], it was proposed that syntaxin 1A was necessary for G-protein regulation of the N-type channel [32]. Further investigations