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Bipartite syntaxin 1A interactions mediate $Ca_V2.2$ calcium channel regulation

Jonathan N. Davies, Scott E. Jarvis, Gerald W. Zamponi *

Hotchkiss Brain Institute, Department of Physiology and Pharmacology, University of Calgary, 3330 Hospital Dr. NW, Calgary AB, Canada T2N 4N1

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ABSTRACT

Functional interactions between syntaxin 1A and Ca_V2 calcium channels are critical for fast neurotransmitter release in the mammalian brain, and coexpression of syntaxin 1A with these channels not only regulates channel availability, but also promotes G-protein inhibition. Both the syntaxin 1A C-terminal H3 domain, and N-terminal Ha domain have been shown to interact with the $Ca_V2.2$ channel synprint region, suggesting a bipartite model of functional interaction, however the molecular determinants of this interaction have not been closely investigated. We used in vitro binding assays to assess interactions of syntaxin 1A truncation mutants with $Ca_V2.2$ synprint and $Ca_V2.3$ II–III linker regions. We identified two distinct interactions between the $Ca_V2.2$ synprint region and syntaxin 1A: the first between C-terminal H3c domain of syntaxin 1A and residues 822–872 of $Ca_V2.2$; and the second between the N-terminal 10 residues of the syntaxin 1A Ha region and residues 718–771 of $Ca_V2.2$. The N-terminal syntaxin 1A fragment also interacted with the $Ca_V2.3$ II–III linker. We then performed whole cell patch clamp recordings to test the effects of a putative interacting syntaxin 1A N-terminus peptide with $Ca_V2.2$ and $Ca_V2.3$ channels in a recombinant expression system. A YFP-tagged peptide corresponding to the N-terminal 10 residues of the syntaxin 1A Ha domain was sufficient to allosterically inhibit both $Ca_V2.2$ and $Ca_V2.3$ channel function but had no effect on G-protein mediated inhibition. Our results support a model of bipartite functional interactions between syntaxin 1A and $Ca_V2.2$ channels and add accuracy to the two putative interacting domains, consistent with previous studies. Furthermore, we highlight the syntaxin 1A N-terminus as the minimal determinant for functional regulation of $Ca_V2.2$ and $Ca_V2.3$ channels.

1. Introduction

The Ca_V2 family of voltage-dependent calcium channels plays a critical role in calcium-dependent, fast neurotransmitter release in the mammalian presynapse [1–4]. This process is tightly regulated, with the synaptic protein syntaxin 1A playing a central role in regulating Ca_V2 channel function bidirectionally, first by inhibiting the channel in the absence of a docked vesicle, and second, by permitting calcium entry only when a mature SNARE complex, primed for neurotransmitter release, is available [5] (for review see [6]). When Ca_V2 channel interactions with syntaxin 1A are perturbed [7] or abolished [8], neurotransmission is compromised.

Syntaxin 1A was first found to associate with the $Ca_V2.2$ channel at a motif on the channel II–III linker, coined the *syn*aptic *pro*tein *int*eraction (synprint) site [9,10]. These, and later studies, implicated the syntaxin 1A C-terminal H3 domain in this interaction [7,11,12]. The $Ca_V2.3$ channel interaction with syntaxin 1A was also thought to take place within the H3 domain [13], because binding and inhibition is lost following cleavage of syntaxin 1A by BoNTC1 [14]. It was argued that the H3 domain interaction was partially disrupted when syntaxin 1A switched from its 'closed' to 'open' configuration, becoming available for four-helical SNARE assembly, but remaining anchored to the channel. However, syntaxin 1A was later found able to bind multiple adjacent synprint motifs [15,16], with the N-terminal Ha region also interacting with synprint and causing functional inhibition of $Ca_V2.2$ [16–18]. This suggested that syntaxin 1A might mediate bipartite interactions with Ca_V2 channels [11,16].

Although several studies have examined the molecular determinants of interaction of either syntaxin 1A or the $Ca_V2.2$ synprint region in isolation, the putative sites of interaction and functional implications of a bipartite model have not been robustly investigated.

Here, we explore the molecular determinants of both syntaxin 1A and the $Ca_V2.2$ synprint region. Our results refine the putative location of two distinct sites of interaction between syntaxin 1A and the $Ca_V2.2$ synprint region. We also show that the N-terminal 10 residues of syntaxin 1A are sufficient to allosterically inhibit both $Ca_V2.2$ and $Ca_V2.3$, suggesting that channel inhibition and anchoring occur at two distinct sites.

* Corresponding author. Address: Department of Physiology and Pharmacology, University of Calgary, 3330 Hospital Dr. NW, Calgary AB, Canada T2N 4N1. Fax: +1 403 210 8106.
E-mail addresses: daviej@ucalgary.ca (J.N. Davies), sejarvis@ucalgary.ca (S.E. Jarvis), zamponi@ucalgary.ca (G.W. Zamponi).

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2. Materials and methods

2.1. Molecular biology

The $Ca_V2.2$ synprint region was subcloned into pTrcHisC to add an N-terminal Xpress epitope as previously described [17] then truncated from the C-terminus in approximately 50 residue intervals to produce coding sequences corresponding to (full length channel) residues 718–919 (ST4), 718–869 (ST3), 718–820 (ST2) and 718–767 (ST1) using site-directed mutagenesis [17]. The $Ca_V2.3$ II–III linker was subcloned into pTrcHisA at restriction sites 5′XhoI, 3′KpnI to add an N-terminal Xpress epitope. Cytoplasmic syntaxin 1A, cloned into pGex-4T-3 to add a GST-tag, and truncation mutants producing coding sequences 1–228, 1–183, 1–158, 1–109, and 1–69, are previously described [17,19]. GST-syntaxin 1A truncations within the Ha domain producing coding sequences for amino acids 1–59, 1–50, 1–40, 1–30, 1–20 and 1–10 were created by site-directed mutagenesis. The N-terminal 10 residues of syntaxin 1A M-KDRTQELRTA-K were scrambled to M-TLKATRRQDE-K as a negative control for $Ca_V2.2$ and Cav2.3 II–III linker binding specificity, created by annealing sense (5′-AAT TCC ATG ACC CTC AAG GCC ACG CGC CGA CAG GAC GAG AAG C-3′) and antisense (5′-GTA CTG GGA GTT CCG GTG CGC GGC TGT CCT GCT CTT CGA GCT-3′) oligonucleotides of the cut insert, at 50 °C for 30 min, then ligated directly into pGEX-5X-3 at restriction sites 5′EcoRI, 3′XhoI.

For confirmation of recombinant colocalization and electrophysiological analysis, the N-terminal 10 residues of syntaxin 1A were cloned into pEYFP-N at restriction sites 5′EcoRI, 3′XhoI to produce a peptide with a C-terminal YFP-tag (YFP-1A10), using Taq DNA polymerase (Invitrogen, Burlington, ON) and PCR steps as per manufacturer's recommendations.

2.2. In vitro binding assays

Protein lysate preparation and GST in vitro binding assays, PAGE and Western blotting were performed as previously described [17]. Protein interaction was confirmed by probing for Xpress-Ca_V2 using an anti-Xpress 1° antibody, then a HRP-conjugated anti-mouse 2° antibody (both Invitrogen, Burlington, ON). Xpress-tagged Ca_V2 proteins were visualized using standard ECL detection methods, developed and fixed. Blots were repeated a minimum of three times.

2.3. Electrophysiology

Characterization of $Ca_V2.2$ and $Ca_V2.3$ channels was conducted by cotransfecting rat cDNAs encoding their α_1, α_2-δ and β_{1b} subunits (3 μg each) into tsA-201 cells. Wildtype syntaxin 1A effects were investigated by cotransfection with rat full length syntaxin 1A cDNA (3 μg). The functional consequences syntaxin 1A N-terminal interaction was explored by coexpressing YFP-1A10 (5 μg). Cell culture and transfection techniques for all conditions are previously described [20]. Whole cell patch clamp recordings were conducted using external solution containing 20 mM $BaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 40 mM tetraethylammonium-chloride, 87.5 mM CsCl, 10 mM glucose (pH 7.7 with tetraethylammonium-OH) and internal solution containing 108 mM cesium-methanesulfonate, 4 mM $MgCl_2$, 9 mM EGTA, and 9 mM HEPES (pH 7.2 with CsOH). Cells expressing a GFP or YFP marker were selected for patching. Data was acquired using an Axopatch 200B amplifier running pClamp 9.0 software (Axon Instruments, Sunnyville, CA). Currents were low pass-filtered at 1 kHz and digitized at 10 kHz. Series resistance was compensated to 80%. Currents smaller than 80 ρA and larger than 2 nA were excluded from the data set.

Current–voltage relations for $Ca_V2.2$ and $Ca_V2.3$ were recorded by stepping to a test pulse of −60 mV to +60 mV from a holding potential of −100 mV. $Ca_V2.2$ steady-state inactivation data were recorded using a slow inactivation protocol as described previously by Degtiar et al. [21]. Currents were evoked at +10 mV for 20 ms before and immediately after a 30 s conditioning prepulse, applied at 10 mV increments from −100 mV to +10 mV. Channel recovery was promoted by holding the membrane at −100 mV for 60 s between sweeps. $Ca_V2.3$ steady-state inactivation data were recorded using a fast inactivation protocol (a 1500 ms conditioning prepulse applied at 10 mV increments from −100 mV to +10 mV). The steady-state inactivation data were fitted in Prism 5 (Graphpad, La Jolla, CA) using a modified Boltzmann equation. Voltage-dependence of inactivation (Vh) data were extracted from individual cell curve fit values calculated in SigmaPlot (Systat, San Jose, CA).

The ability of the N-terminal 10 residues of syntaxin 1A (YFP-1A10) or wildtype syntaxin 1A to elicit tonic G-protein inhibition of $Ca_V2.2$ and $Ca_V2.3$ channels was assessed as a ratio of peak current amplitude after (+PP) and 200 ms before (−PP) a strong depolarizing prepulse of +150 mV for 50 ms. Peak current amplitude was obtained from a test pulse of +10 mV for 15 ms.

Data analysis and offline leak subtraction was completed with Clampfit 9.0 (Axon Instruments, Sunnyville, CA). All statistical analysis was performed in Prism 5 (Graphpad, La Jolla, CA), using two-tailed unpaired *t*-test. Significance was taken as $p < 0.05$. All values are reported as mean ± standard error.

3. Results

3.1. Syntaxin 1A N- and C-terminal domains interact with distinct $Ca_V2.2$ synprint motifs

We previously demonstrated that full length syntaxin 1A and truncations removing domains H3c (228–268), H3b (183–268), H3a (158–268), Hc (109–268), Hb (69–268) interact with the $Ca_V2.2$ synprint region [17]. Here, we extended our work by assessing interactions between these and a further round of syntaxin 1A Ha domain truncation mutants (59, 50, 40, 30, 20 and 10) and C-terminal $Ca_V2.2$ synprint truncations removing residues 922–965 (ST4), 872–965 (ST3), 822–965 (ST2) and 771–965 (ST1; Fig. 1A). Interactions were determined by probing for Xpress-$Ca_V2.2$ synprint fragments that bound to GST-immobilized syntaxin 1A fragments in vitro. All syntaxin 1A truncations strongly interacted with ST4 (Fig. 1A, top panel) and ST3 (Fig. 1A, second top panel). The strong full length syntaxin 1A interactions with ST2 (Fig. 1A, second bottom panel) and ST1 (Fig. 1A, bottom panel) were weakened (1A228), and then lost (1A183, 1A158, 1A109) as successive syntaxin 1A domains were removed. This loss of binding suggests a first interaction site between the C-terminal H3c domain of syntaxin 1A and $Ca_V2.2$[822–872].

ST2 regained weak interactions within the Ha domain (1A69, 1A59, 1A50, 1A40, 1A30, 1A20) that recovered strength at the N-terminus (1A10), whereas ST1 regained strong interactions further C-terminal in the Ha domain (1A59, 1A50, 1A40, 1A30). This gain of binding suggests a second site of interaction between as little as the N-terminal 10 residues of the syntaxin 1A Ha region and $Ca_V2.2$[718–771].

To rule out the possibility of non-specific antibody interactions, pure Xpress-synprint protein lysate was run alongside the same protein bound to GST-syntaxin 1A (Fig. 1B). Reactivity in both lanes suggested a positive match for Xpress-synprint. Neither pure GST-syntaxin 1A nor GST protein lysate were detected by the anti-Xpress antibody.

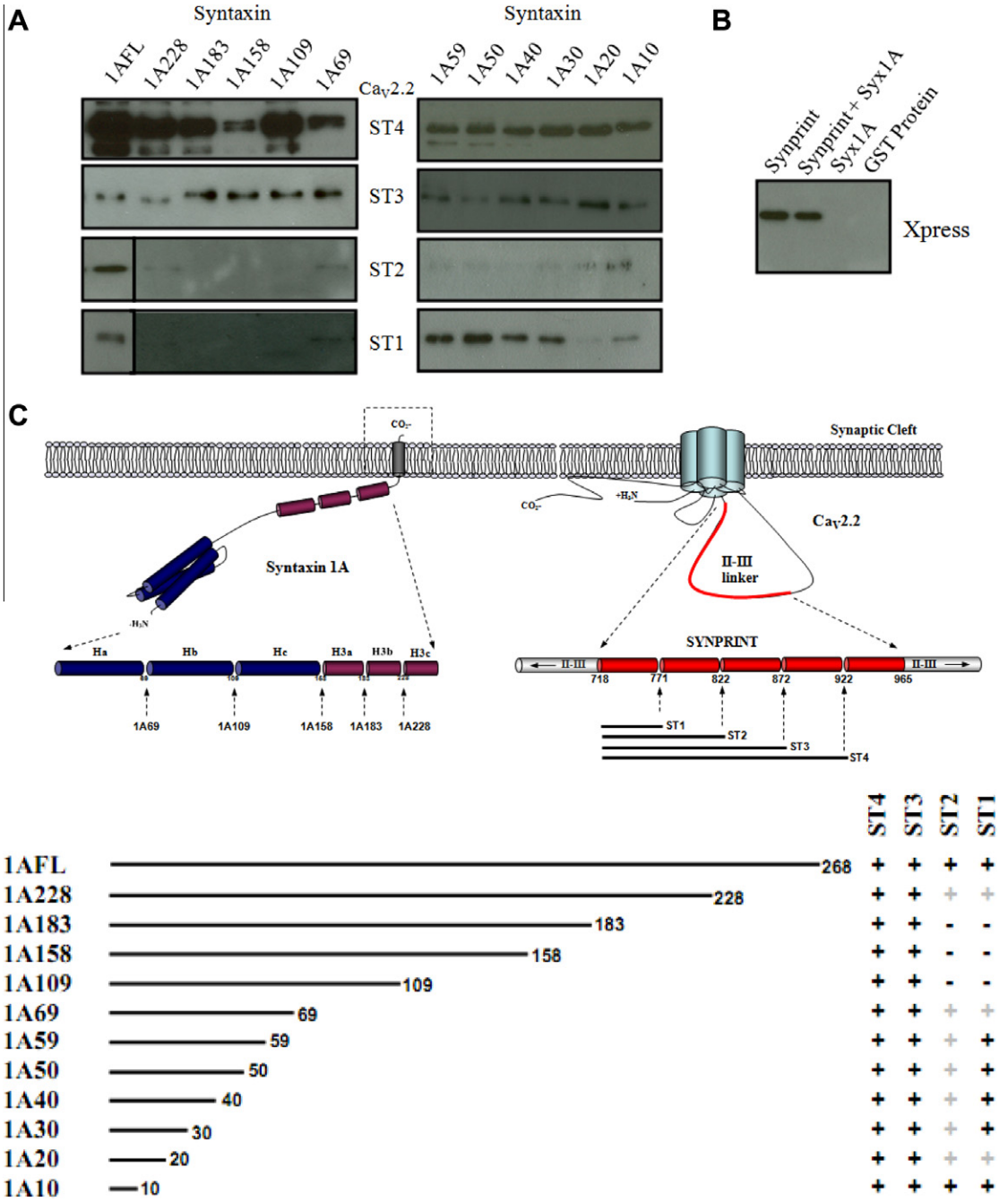

Fig. 1. Only the syntaxin 1A Ha domain retains strong interaction with $Ca_V2.2$ truncations ST2 and ST1. (A) $Ca_V2.2$ synprint protein truncations removing residues 922–965 (ST4), 872–965 (ST3), 822–965 (ST2) and 771–965 (ST1) were probed with an anti-Xpress antibody to demonstrate in vitro binding with syntaxin 1A truncations removing domains H3c (228–268), H3b (183–268), H3a (158–268), Hc (109–268), Hb (69–268), then further Ha domain truncations (59, 50, 40, 30, 20 and 10). (B) Control blot showing side-by-side comparison of Xpress-synprint (32.6 kDa) protein alone versus bound to GST-syntaxin 1A. Negative controls GST-syntaxin 1A (57.6 kDa) and pure GST protein (26.1 kDa) proteins did not interact with the anti-Xpress antibody. Blots were repeated a minimum of three times. (C) The schematic diagram and table summarize which $Ca_V2.2$ synprint truncations (top right, red; ST4, 3, 2 and 1) interacted strongly (+), weakly (+) or not at all (−) with syntaxin 1A truncations (top left, blue and purple; 1AFL, 1A−228, −183, −158, −109, −69, −59, −50, −40, −30, −20 and −10). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. The N-terminal 10 residues of syntaxin 1A interact with and inhibit $Ca_V2.2$ but do not mediate tonic G-protein inhibition

Having identified the 10 residue N-terminus of syntaxin 1A (1A10) as one minimal structural determinant of interaction, we investigated its functional effects on $Ca_V2.2$. We created a YFP-tagged syntaxin 1A N-terminal peptide, and after confirming colocalization with wildtype $Ca_V2.2$ channel complexes in tsA-201 cells (data not shown), we performed whole cell patch clamp recordings to test the effects of the syntaxin 1A N-terminal interaction with $Ca_V2.2$ channels in a recombinant expression system.

$Ca_V2.2$ peak current (−579.8 ± 129.3 pA) and current density (−29.5 ± 6.1 pA/pF) were significantly reduced with syntaxin 1A coexpression (−202.5 ± 31.8 pA, $p < 0.05$; −10.4 ± 1.5 pA/pF; $p < 0.05$). Syntaxin 1A also caused a ~6 mV hyperpolarizing shift

in half-inactivation (Vh; −69.9 ± 0.7 mV; $p < 0.05$) relative to $Ca_V2.2$ (−63.4 ± 0.4 mV; Fig. 2A).

Peak current (−579.8 ± 129.3 pA) and current density (−29.5 ± 6.1 pA/pF) were unchanged with YFP-1A10 coexpression (−540.4 ± 135.2 pA; −25.4 ± 7.6 pA/pF; Fig. 2A). YFP-1A10 caused a ~5 mV hyperpolarizing shift in the half-inactivation potential (Vh; −67.9 ± 0.6 mV, $p < 0.05$; Fig. 2A) compared to $Ca_V2.2$ alone (−63.4 ± 0.4 mV). These data indicate that the truncated YFP-tagged syntaxin 1A N-terminus is able to bind to the channel in a cellular environment and regulate its function.

$Ca_V2.2$ channels are subject to an additional layer of modulation by interactions with G-protein Gβγ subunits that promote tonic inhibition of channel activity [19,22,23]. Predictably, syntaxin 1A elicited this effect ($I_{(+PP/-PP)}$ = 1.4 ± 0.4; $p < 0.05$; Fig. 2B) but YFP-1A10 did not (1.1 ± 0.03). This suggests that syntaxin 1A N-terminus does not mediate tonic G-protein inhibition of $Ca_V2.2$.

3.3. The N-terminal 10 residues of syntaxin 1A interact with and directly inhibit the $Ca_V2.3$ II–III linker but do not mediate tonic G-protein inhibition

While syntaxin 1A does interact with and regulate $Ca_V2.3$ channels [13,24], to our knowledge, the molecular determinants of this interaction have not previously been investigated. As such, we tested our GST-syntaxin 1A constructs for in vitro binding interactions with the Xpress-$Ca_V2.3$ II–III linker. Syntaxin 1A and all truncations interacted with the $Ca_V2.3$ II–III linker (Fig. 3A). A GST-tagged scrambled peptide control based on 1A10 did not interact with the Xpress-$Ca_V2.3$ II–III linker.

$Ca_V2.3$ peak current amplitude (−713.7 ± 245.9 pA) and current density (−40.5 ± 12.4 pA/pF) were unchanged with syntaxin 1A coexpression (−336.8 ± 97.6 pA; −26.4 ± 9.7 pA/pF; Fig. 3B) or YFP-1A10 (−610.1 ± 236.6 pA; −33.6 ± 13.3 pA/pF). Syntaxin 1A caused a ~13 mV hyperpolarizing shift in half-inactivation (Vh; −70.2 ± 2.4 mV, $p < 0.05$; Fig. 3B) relative to $Ca_V2.3$ (−57.1 ± 1.8 mV). YFP-1A10 elicited a hyperpolarizing shift of ~7 mV (−64.3 ± 1.5 mV, $p < 0.05$; Fig. 3B) relative to $Ca_V2.3$ alone. Neither syntaxin 1A ($I_{(+PP/-PP)}$ = 0.9 ± 0.05) or YFP-1A10 ($I_{(+PP/-PP)}$ = 0.8 ± 0.03; Fig. 3C) mediated tonic G-protein inhibition of $Ca_V2.3$ channels.

4. Discussion

This study sought to determine whether syntaxin 1A regulates $Ca_V2.2$ channel function through bipartite functional interactions. We found evidence supporting two distinct interactions: the first between the syntaxin 1A N-terminus and $Ca_V2.2$[718–771]; and the second between the syntaxin 1A H3 domain and $Ca_V2.2$

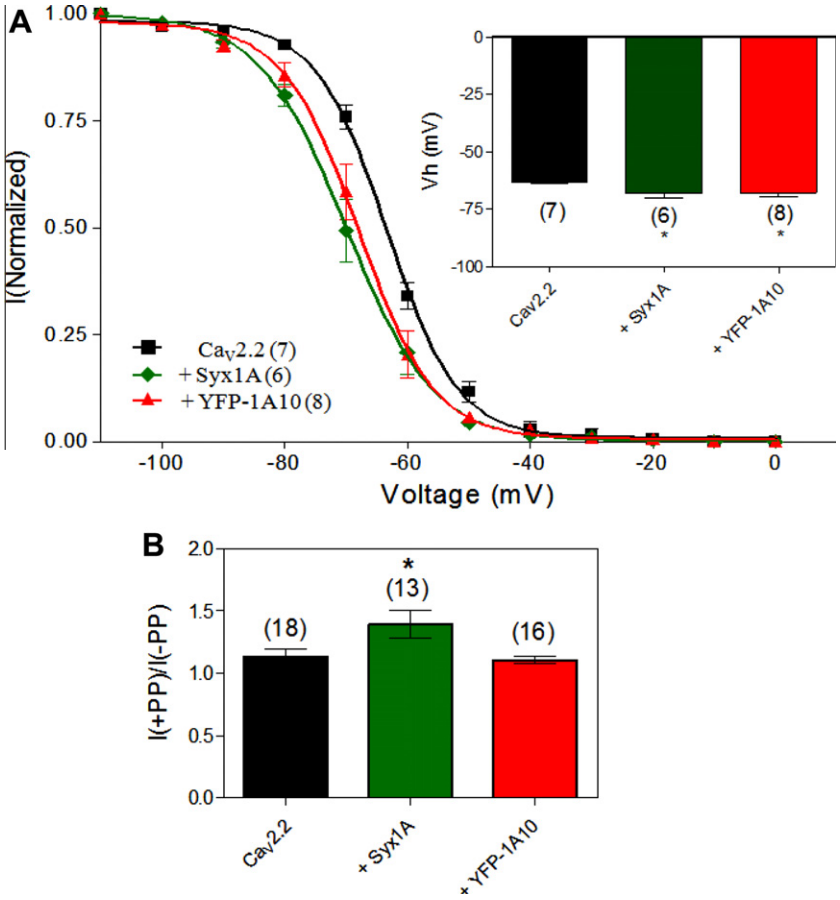

Fig. 2. The N-terminal 10 residues of syntaxin 1A directly inhibit $Ca_V2.2$ but do not mediate tonic G-protein inhibition (A) Half-inactivation potentials (Vh) were derived from the steady-state inactivation curve. Syntaxin 1A (green diamond; −69.9 ± 0.7 mV, $n = 8$, $p < 0.05$) caused a ~6 mV hyperpolarizing shift in the voltage dependence of inactivation relative $Ca_V2.2$ (black square; −63.4 ± 0.4 mV, $n = 7$). Syntaxin 1A's The N-terminal 10 residues (YFP-1A10; red triangle) caused a ~5 mV hyperpolarizing shift (−67.9 ± 0.6 mV, $n = 9$, $p < 0.05$) relative to wildtype. Rat $\alpha_2\delta_1$ and β_{1b} were coexpressed with all conditions. Statistical significance was assessed using unpaired two-tailed t-test with $p < 0.05$. (B) Tonic G-protein inhibition of $Ca_V2.2$ was assessed as a ratio of peak current amplitude after (+PP) and before (−PP) a 50 ms prepulse of +150 mV. Peak current amplitude decreased with coexpression of syntaxin 1A leading to a larger $I_{(+PP/-PP)}$ (1.4 ± 0.4, $n = 13$, $p < 0.05$) relative to $Ca_V2.2$ alone (1.1 ± 0.1, $n = 18$), following a strong depolarizing prepulse. YFP-1A10 did not enhance $I_{(+PP/-PP)}$ (1.1 ± 0.03, $n = 16$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

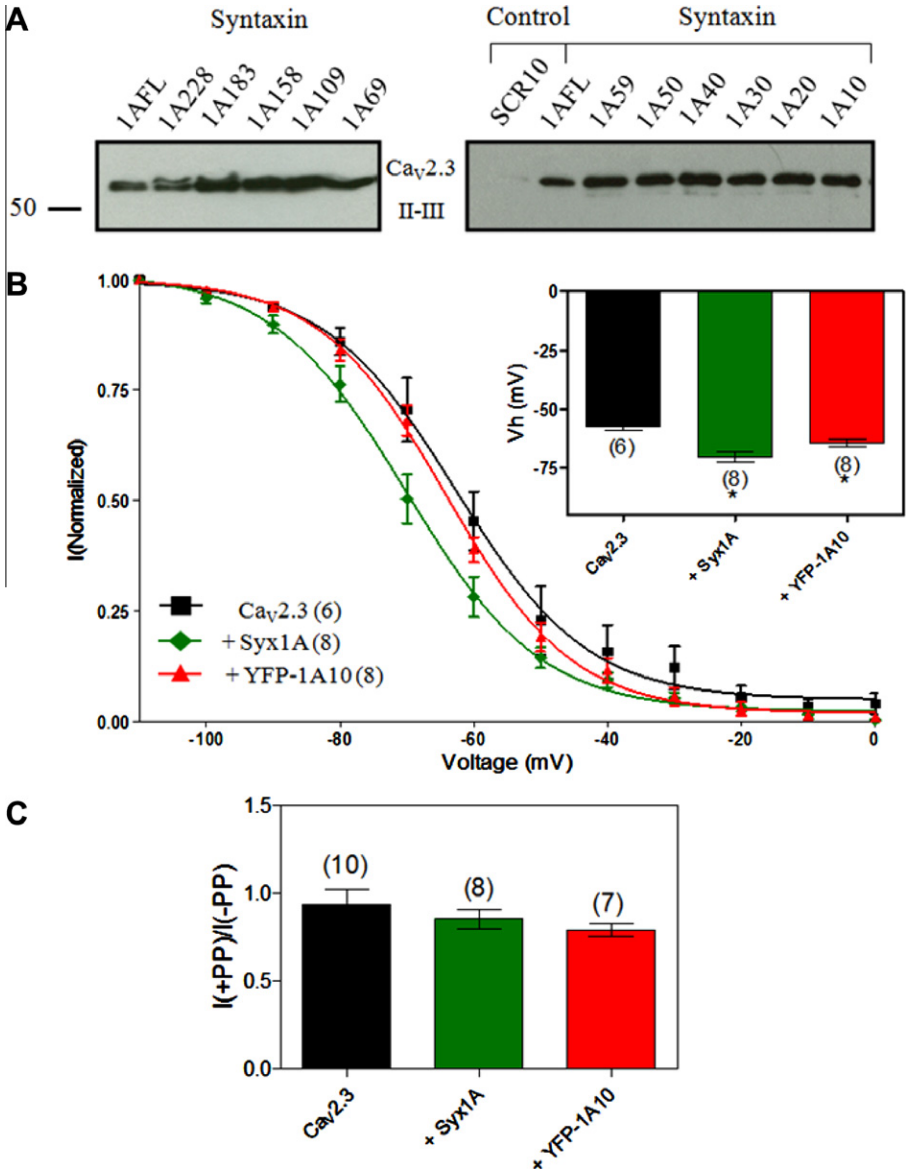

Fig. 3. The N-terminal 10 residues of syntaxin 1A interact with and directly inhibit the $Ca_V2.3$ II–III linker but do not mediate tonic G-protein inhibition.(A) *In vitro* binding assay in which GST-immobilized syntaxin 1A truncations were incubated with Xpress-tagged $Ca_V2.3$ II–III. The $Ca_V2.3$ II–III linker retained interaction with syntaxin 1A truncations: H3c (228–268), H3b (183–268), H3a (158–268), Hc (109–268), Hb (69–268), then within further truncations within the Ha domain (59, 50, 40, 30, 20 and 10). A GST-tagged scrambled peptide control of the N-terminal 10 residues of syntaxin 1A (M-TLKATRRQDE-K) was used as a control to confirm binding specificity. The Xpress-$Ca_V2.3$ II–III linker (55.4 kDa) interacted with GST-tagged 1A10 but not the GST-tagged scrambled peptide. Western blots were probed with an anti-Xpress antibody (Invitrogen, Burlington, ON). Blots were repeated a minimum of three times. (B) Half inactivation potentials (Vh) were obtained from the steady-state inactivation curve. Coexpression of syntaxin 1A (green diamond; -70.2 ± 2.4 mV, $n = 7$, $p < 0.05$) caused a ~13 mV hyperpolarizing shift in the voltage dependence of inactivation relative to $Ca_V2.3$ (black square; -57.1 ± 1.8 mV, $n = 6$). YFP-1A10 (red triangle; -64.3 ± 1.5 mV, $n = 8$; $p < 0.05$) caused a hyperpolarizing shift of ~7 mV. Rat $\alpha_2\delta_1$ and β_{1b} were coexpressed with all conditions. Statistical significance was assessed using unpaired two-tailed *t*-test with $p < 0.05$. (C) Tonic G-protein inhibition of $Ca_V2.3$ was assessed as a ratio of peak current amplitude after (+PP) and before (−PP) a 50 ms prepulse of +150 mV. $Ca_V2.3$ peak current amplitude was unaffected by the prepulse, resulting in an $I_{(+PP/-PP)}$ of 0.9 ± 0.08 ($n = 10$), which was unchanged after coexpression with syntaxin 1A (0.9 ± 0.05, $n = 8$) or YFP-1A10 (0.8 ± 0.03, $n = 7$). Statistical significance was assessed using unpaired two-tailed *t*-test with $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

[822–872]. We then demonstrated that the N-terminal 10 residues of syntaxin 1A are able to interact with and allosterically inhibit $Ca_V2.2$ and $Ca_V2.3$ channel activity.

Our finding of a strong interaction between the N-terminal 10 residues of syntaxin 1A and $Ca_V2.2$[718–771] is consistent with our earlier findings that interaction persisted between synprint and the syntaxin 1A Ha domain [17]. This interaction likely occurs by virtue of a specific motif rather than a cluster of positive charges. Subsequent deletions within the $Ca_V2.2$ synprint region narrowed this interaction down to the N-terminal 54 residues of synprint: $Ca_V2.2$[718–771]. This interaction is consistent with Yokoyama et al. [16] who showed interaction between $Ca_V2.2$[718–789] (along with a second, distinct interacting site between $Ca_V2.2$[860–873]), and also Sheng et al. [10] who identified a weakly interacting "subsite" from residues $Ca_V2.2$[718–773]. Together these data support the notion that the syntaxin 1A N-terminus interacts with the upstream portion of the synprint region. The fact that as few as 10 amino acids of syntaxin 1A were not only capable of interacting with both channel subtypes examined, but also to regulate them at a functional level is surprising, especially given that C-terminal cleavage by botulinum toxin C1 abolishes syntaxin 1A mediated effects on channel gating [14]. The effects

of the 1A10 peptide are possibly masked when forming part of the whole syntaxin 1A molecule. YFP-1A10 did not induce G-protein inhibition of the channels, supporting our earlier observation that syntaxin 1A's H3a and H3b domains promote Gβγ binding and colocalization [17].

It is also interesting to note that C-terminal interaction between the syntaxin 1A H3c subdomain and $Ca_V2.2$[822–872], which was discovered when syntaxin 1A interaction weakened with the removal of the H3c domain concurrent with 143 residues (ST2; 822–872) or 194 residues (ST1; 771–822) of the synprint region, an interaction that was lost completely as further C-terminal syntaxin 1A domain deletions were made. Although the existence of an N-terminal interaction described earlier should have masked this second interaction, it is possible that a truncated syntaxin 1A protein may have been folded so as to obscure the N-terminus. The literature suggests our putative C-terminal interaction between the syntaxin 1A H3c domain and $Ca_V2.2$[822–872] is highly congruent with previous studies [10,11,15]. Furthermore, our results narrow the determinants of syntaxin 1A interaction down to the H3c subdomain within H3. Sheng et al. [10] found the H3 domain interacted with a motif spanning residues $Ca_V2.2$[773–859] and similarly lost the C-terminal interaction with further truncations. Rettig et al. [15] found syntaxin 1A H3 interactions in three overlapping segments, all containing the common sequence $Ca_V2.2$[832–859]. This finding was also independently recapitulated by Yokoyama et al. [16] who concluded that $Ca_V2.2$[849–858] was the minimal determinant of interaction. Taken together, our findings support a bipartite model of interaction between these two proteins.

We also investigated the molecular determinants of interaction between syntaxin 1A and $Ca_V2.3$ which have been largely unexplored (but see [13,24]). In our study, all syntaxin 1A truncations including the N-peptide interacted with the $Ca_V2.3$ II–III linker. The syntaxin 1A N-peptide binding with both $Ca_V2.2$ and $Ca_V2.3$ is suggestive of a conserved binding motif in both channels.

Recently, both the syntaxin 1A N-terminus and the anchored C-terminus were found to associate with the sec1/munc-18 like (SM) protein, munc18-1 [25], and evidence is emerging that both proteins are required for efficient neurotransmitter release [26,27]. It is yet unclear whether the syntaxin 1A N-terminus interaction with munc18-1 serves to facilitate syntaxin 1A's transition from 'closed' to 'open' state [25], stabilize syntaxin 1A's closed conformation [28] or control its access to other synaptic binding partners [29]. Munc18-1 also binds with high affinity to the $Ca_V2.2$ II–III loop, possibly serving as a bridge for v-SNARE synaptotagmin1 [30]. One plausible scenario is that in the vesicle priming phase, munc18-1 competes syntaxin 1A's N-terminus away from the $Ca_V2.2$ (or $Ca_V2.3$) channel and in doing so, not only aids its switch from 'closed' to 'open' conformation (allowing four-SNARE alignment), but also releases direct channel inhibition. It will be interesting to see how the intricate, overlapping roles of motifs on this and other synaptic proteins develop, and contribute to the complex interplay that is SNARE-mediated exocytosis.

In conclusion, this study supports the notion that syntaxin 1A N- and C-terminal domains mediate bipartite interactions with $Ca_V2.2$ (and possibly $Ca_V2.3$), and has identified the syntaxin 1A N-terminus as responsible for allosterically promoting $Ca_V2.2$ and $Ca_V2.3$ channel inactivation.

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