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Ca^{2+}-dependent and -independent interactions of the isoforms of the α_{1A} subunit of brain Ca^{2+} channels with presynaptic SNARE proteins

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ABSTRACT **Fast neurotransmission requires that docked synaptic vesicles be located near the presynaptic N-type or P/Q-type calcium channels. Specific protein–protein interactions between a synaptic protein interaction (synprint) site on N-type and P/Q-type channels and the presynaptic SNARE proteins syntaxin, SNAP-25, and synaptotagmin are required for efficient, synchronous neurotransmitter release. Interaction of the synprint site of N-type calcium channels with syntaxin and SNAP-25 has a biphasic calcium dependence with maximal binding at 10–20 μM. We report here that the synprint sites of the BI and rbA isoforms of the α_{1A} subunit of P/Q-type Ca^{2+} channels have different patterns of interactions with synaptic proteins. The BI isoform of α_{1A} specifically interacts with syntaxin, SNAP-25, and synaptotagmin independent of Ca^{2+} concentration and binds with high affinity to the C2B domain of synaptotagmin but not the C2A domain. The rbA isoform of α_{1A} interacts specifically with synaptotagmin and SNAP-25 but not with syntaxin. Binding of synaptotagmin to the rbA isoform of α_{1A} is Ca^{2+}-dependent, with maximum affinity at 10–20 μM Ca^{2+}. Although the rbA isoform of α_{1A} binds well to both the C2A and C2B domains of synaptotagmin, only the interaction with the C2A domain is Ca^{2+}-dependent. These differential, Ca^{2+}-dependent interactions of Ca^{2+} channel synprint sites with SNARE proteins may modulate the efficiency of transmitter release triggered by Ca^{2+} influx through these channels.**

Neurotransmitter release from specialized active zones in presynaptic terminals is a critical step in synaptic transmission. Release of synaptic vesicles containing neurotransmitter is triggered within 200 μs of depolarization of the nerve terminal by influx of Ca^{2+} through voltage-gated Ca^{2+} channels that raises the local concentration of Ca^{2+} from a basal level of 100 nM to more than 100 μM (1–4). Evidence for an integral role for the N-type Ca^{2+} channel in neurotransmitter release at peripheral and central synapses has been provided by both immunocytochemical and pharmacological experiments (5–8). However, P/Q-type Ca^{2+} channels are present in high density at central synapses (9), and transmitter release primarily requires P/Q-type channels, with N-type channels playing a secondary role (10–13). Ca^{2+} channels are complexes of a pore-forming α_1 subunit with auxiliary $\alpha_2\delta$, β, and, in skeletal muscle, γ subunits (14). N-type Ca^{2+} channels contain α_{1B} subunits whereas P/Q-type Ca^{2+} channels are thought to contain α_{1A} subunits (15, 16).

The synaptic vesicle SNARE proteins synaptotagmin and synaptobrevin and the synaptic plasma membrane SNARE proteins SNAP-25 and syntaxin form a tight complex at the presynaptic plasma membrane that is implicated in the docking of vesicles and/or exocytosis of neurotransmitter during synaptic transmission (17–19). Synaptotagmin is thought to serve as the Ca^{2+} sensor for initiation of fast transmitter release (17, 19). Antibodies against syntaxin and synaptotagmin coimmunoprecipitate N-type and P/Q-type Ca^{2+} channels from solubilized brain membranes, consistent with formation of a specific complex containing these proteins *in situ* (20–22). These binding interactions involve a synaptic protein interaction (synprint) site in the intracellular loop between domains II and III ($L_{II\text{-}III}$) of the α_{1A} and α_{1B} subunits of these Ca^{2+} channels (23–27). Evidently, the Ca^{2+} channel itself is an integral component of the protein complex involved in the neurotransmitter release.

A functional role for these protein–protein interactions in synaptic transmission was demonstrated by electrophysiological studies of synapses between superior cervical ganglion cells and between nerve and muscle cells in cell culture. Introduction of a peptide containing the synprint site of the N-type Ca^{2+} channel into the presynaptic cells resulted in inhibition of fast synaptic transmission (28, 29) and increased paired-pulse facilitation and late excitatory postsynaptic potentials after a tetanic stimulation (28). In addition, interaction with SNARE proteins functionally modulates both N-type and P/Q-type channels when coexpressed in *Xenopus* oocytes (30, 31). These data suggest that interaction between synaptic proteins and the N-type and P/Q-type Ca^{2+} channel serves to position docked synaptic vesicles near sites of Ca^{2+} entry to allow efficient exocytosis and to modulate channel function.

The interaction of N-type Ca^{2+} channels with syntaxin and SNAP-25 is Ca^{2+}-dependent, with maximal binding occurring at Ca^{2+} concentrations of 10–20 μM (24), and is regulated by protein phosphorylation (32). The synprint site of N-type channels competes with synaptotagmin for binding to syntaxin (26). The α_{1A} subunit of P/Q-type Ca^{2+} channels exists in two isoforms, designated BI and rbA when they were initially cloned (33, 34), which differ in the amino acid sequence of $L_{II\text{-}III}$ and are differentially localized in rat and rabbit brain (35). These two isoforms bind SNAP-25 similarly but differ in their affinity for syntaxin (25). Because P/Q-type Ca^{2+} channels containing the α_{1A} subunit are primarily responsible for neurotransmitter release at central synapses, it is important to determine the interactions of the two isoforms of α_{1A} with synaptotagmin, the putative Ca^{2+} sensor for transmitter release, and to examine the Ca^{2+} dependence of their interaction with syntaxin, SNAP-25, and synaptotagmin. In this report, we describe differential Ca^{2+}-dependent interactions of synaptotagmin with the rbA and BI isoforms of α_{1A} and compare the interactions of the SNARE proteins with these two α_{1A} isoforms and with α_{1B}. The results show that these three isoforms of presynaptic Ca^{2+} channels have quite different

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Abbreviations: GST, glutathione *S*-transferase; $L_{II\text{-}III}$, intracellular loop between domains II and III of the Ca^{2+} channel α_1 subunit; S25, SNAP-25; syn, syntaxin; syt, synaptotagmin; synprint, *syn*aptic *pro*tein *inte*raction site; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor.

interactions with SNARE proteins, suggesting that regulation of the synaptic transmission mediated by these channel subtypes may be significantly different.

EXPERIMENTAL PROCEDURES

Materials. The cDNA clone of SNAP-25 (36) was provided by M. Wilson (Scripps Institute, La Jolla, CA). M. Takahashi provided syntaxin and synaptotagmin cDNA (22). T. Snutch provided the cDNAs encoding the α_{1B} subunit of the N-type Ca^{2+} channel (37) and the rbA and BI isoforms of the P/Q-type channel (25, 33). Expression vector pGEX-4T, glutathionine-coated Sepharose 4B beads, and anti-GST antibody were obtained from Pharmacia LKB. Expression vector pTrcHis C was obtained from Invitrogen. T7-Tag monoclonal antibody and the protease-deficient BL26 strain of *Escherichia coli* were obtained from Novagen. The T7 antibody recognizes the sequence containing the six-histidine tag of the fusion proteins generated through subcloning into the pTrcHis C expression vector.

Construction and Isolation of Fusion Proteins. Fusion protein construction and purification were performed as previously described (23–26, 32). To generate recombinant glutathione *S*-transferase (GST)-fusion proteins, syntaxin and SNAP-25 cDNA were amplified by PCR and cloned into the pGEX-4T expression vector. Recombinant GST-synaptotagmin fusion proteins were prepared from expression plasmids in the vector pGEX-2T (Pharmacia) as described (26). These synaptotagmin fusion protein constructs included those containing full-length cytoplasmic domains encoding residues 80–421, referred to as synaptotagmin or syt; the C2A domain containing residues 128–269 (syt-2A); and the C2B domain containing residues 262–385 (syt-2B).

Both the GST-tagged synaptic protein constructs and the His-tagged Ca^{2+} channel constructs were transformed into the protease-deficient BL26 strain of *E. coli*. Induction of fusion protein production was performed with the addition of isopropyl-D-thiogalactopyranoside (0.2 mM final concentration). The fusion proteins were isolated by affinity chromatography by using glutathionine-coated Sepharose 4B beads for GST fusion proteins and by affinity chromatography using nickel-conjugated resin (Qiagen) for the His-tagged fusion proteins. Expressed fusion proteins were released by lysing the cells by mild sonication. Protein concentrations were determined by bicinchoninic acid assay (Pierce). All constructs were verified by DNA sequencing.

Binding Experiments and Immunoblotting. Protein–protein interactions between the synaptic proteins, syntaxin, SNAP-25, and synaptotagmin and the N-type and P/Q-type Ca^{2+} channels were investigated by using GST-tagged fusion proteins as an affinity matrix for the specific binding to the His-tagged Ca^{2+} channel proteins (23–26, 32). Briefly, the GST fusion proteins were bound (at 4°C, 60 min) to glutathione-Sepharose beads (Pharmacia LKB) in PBS containing 0.1% Triton X-100 and 0.4 μM phenylmethanesulfonyl fluoride. After three washes with PBS, the beads with bound GST-tagged fusion proteins were incubated with His-tagged Ca^{2+} channel fusion proteins for 3 h at 4°C. Bound proteins were then eluted from the beads with 15 mM reduced glutathione/50 mM Tris·HCl (pH 8) in a volume of 20 μl. Eluted samples were separated from the beads by centrifugation at 10,000 $\times$ *g* for 1 min. Supernatants (20 μl) were then boiled for 2 min with 10 μl 3$\times$ Tricine sample buffer. Proteins were separated on 10–20% Tricine gradient gels (NOVEX, San Diego) and transferred overnight to 0.2 μm nitrocellulose membranes (Schleicher & Schuell). Specific binding was then visualized by immunoblotting with T7-Tag antibody (1:10,000 dilution) (Novagen) followed by image development with enhanced chemiluminescence (ECL system, Amersham). Concentrations of GST-tagged fusion protein immobilized to Sepharose beads were quantitated either by staining with Ponceau S (Sigma) or by immunoblotting with anti-GST antisera (Pharmacia LKB) after stripping of the nitrocellulose membrane (32).

Ca^{2+} Dependence of Binding. The binding experiments are similar to those described in the previous section, except Ca^{2+} concentration in the buffer was controlled with 5 mM *N*-hydroxyethylethylenediamine-triacetic acid (HEDTA, Sigma) (26). Ca^{2+} ionic concentration and buffer calculations were performed with the MAX chelator program (version 6.62). The buffer for the binding reaction was Tris (15 mM), NaCl (140 mM), and Hepes (50 mM), pH 7.2. The washes were performed with the same Ca^{2+} concentration as in the binding buffer.

RESULTS

Interaction of the rbA and BI Isoforms of α_{1A} with Synaptotagmin. Previous results showed that a His-tagged fusion protein containing the synprint region of α_{1B} binds to GST-syntaxin, GST-SNAP-25, and GST-synaptotagmin specifically (23, 24, 26). In contrast, the synprint peptide from the rbA isoform of α_{1A} binds GST-SNAP-25 but not GST-syntaxin

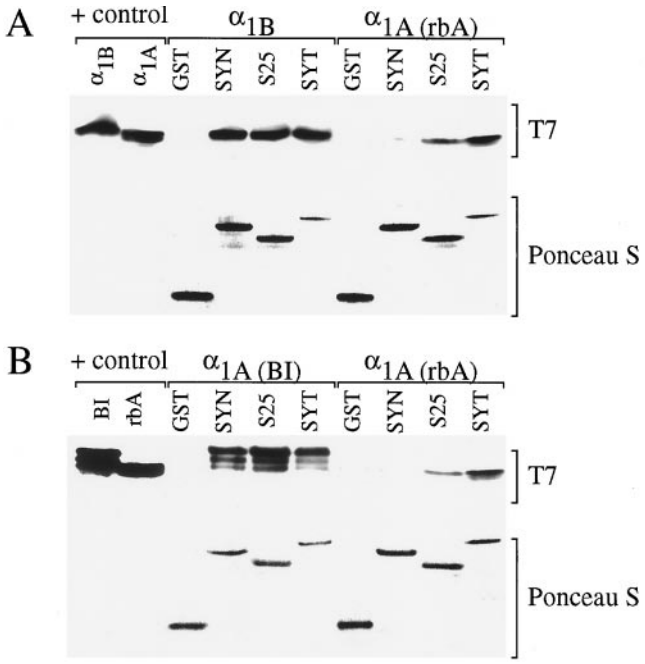

FIG. 1. Binding of α_{1B}, $\alpha_{1A(rbA)}$, and $\alpha_{1A(BI)}$ to SNARE proteins. GST-fusion proteins containing synaptotagmin, syntaxin, SNAP-25, or GST alone (50 pmol) were incubated with glutathione-Sepharose beads. After 60 min, the beads were washed three times with Tris-saline buffer (100 mM Tris·HCl/140 mM NaCl/0.1% Triton X-100, pH 8). Purified His-tagged Ca^{2+} channel fusion proteins from α_{1B} (50 pmol), $\alpha_{1A(rbA)}$ (1.3 nmol), or $\alpha_{1A(BI)}$ (300 pmol) were added to the beads as indicated in the figure. The mixture was then incubated at 4°C for 3 h on a rotating mixer. The Tris-saline buffer containing 15 μM Ca^{2+} was used for the binding and for the washes. The fusion proteins were then eluted with 20 μl elution buffer (15 mM reduced-glutathione in Tris-saline buffer, pH 8). The eluate was boiled in Tricine sample buffer for 2 min, and the proteins were separated on 10–20% Tricine–SDS/PAGE gradient gels and electrophoretically transferred overnight to nitrocellulose. Separated bands were immunoblotted with T7 monoclonal antibody and visualized with the ECL system (Amersham). Concentrations of GST fusion protein in each condition were visualized by staining with Ponceau S. A positive control indicates the level of staining with the T7 mAb for 2.5 pmol of α_{1B}, $\alpha_{1A(rbA)}$, or $\alpha_{1A(BI)}$ added directly to the gel (control). The appearance of multiple bands in this and subsequent figures (e.g., in $\alpha_{1A(BI)}$ samples) is because of minor proteolytic cleavage products of the fusion proteins that retain binding activity.

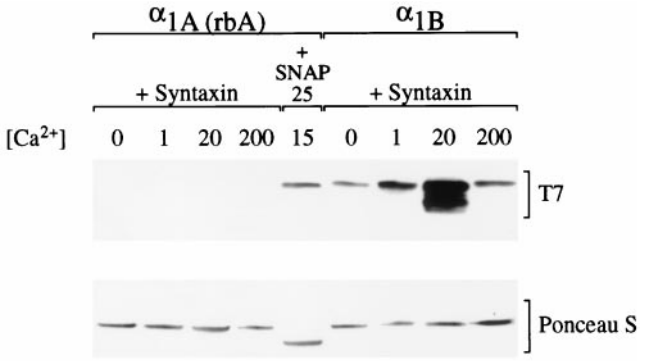

FIG. 2. Ca^{2+}-dependent binding of syntaxin to α_{1B} but not $\alpha_{1A(rbA)}$. Binding of the synprint peptides from α_{1B} and $\alpha_{1A(rbA)}$ to immobilized GST-syntaxin or GST-SNAP-25 was measured as described in *Experimental Procedures* and the legend to Fig. 1 at the indicated concentrations of Ca^{2+}.

whereas the BI isoform of α_{1A} binds well to both GST-syntaxin and GST-SNAP-25 (25). The results shown in Fig. 1*A* confirm that the His-tagged synprint peptide from α_{1B} binds to GST-syntaxin, GST-SNAP-25, and GST-synaptotagmin specifically, as measured by glutathione-Sepharose chromatography and immunoblotting of the eluted complex. Similarly, the BI isoform of α_{1A} binds specifically to GST-syntaxin, GST-SNAP-25, and GST-synaptotagmin (Fig. 1*B*). In contrast, the synprint peptide from the rbA isoform of α_{1A} binds to GST-SNAP-25 and GST-synaptotagmin, but not to GST-syntaxin (Fig. 1). Thus, the synprint region of the rbA isoform of α_{1A} has a distinct pattern of binding to these three SNARE proteins in comparison to α_{1B} or the BI isoform of α_{1A}.

The relative concentrations of Ca^{2+} channel proteins required to produce a similar level of binding (legend of Fig. 1) suggests an approximately 6-fold higher affinity of all three synaptic proteins for α_{1B} than for the BI isoform of α_{1A} and an approximately 25-fold greater binding affinity than the rbA isoform for synaptotagmin or SNAP-25. To provide more quantitative data regarding the relative affinity of α_{1B} and the rbA and BI isoforms of $\alpha_{1A(rbA)}$ for syntaxin, SNAP-25, and synaptotagmin, interactions between the synprint peptides of these Ca^{2+} channels and the three synaptic proteins were measured by using a solid-phase immunoassay (ELISA; ref. 26 and unpublished results). These data support the differences in relative binding affinity observed in these experiments.

Differential Ca^{2+} Dependence of the Interaction of Ca^{2+} Channel Isoforms with Synaptic Proteins. In the absence of Ca^{2+}, the synprint peptide from the rbA isoform of α_{1A} binds to SNAP-25 but not to syntaxin (25). The binding of α_{1B} to syntaxin or to SNAP-25 has a sharp biphasic dependence on Ca^{2+} concentration with peak binding at 10–20 μM (ref. 24; Fig. 2). The binding of the synprint peptide of $\alpha_{1A(rbA)}$ to GST-syntaxin was tested at varying Ca^{2+} concentrations by using the glutathione-Sepharose affinity chromatography assay (Fig. 2). No specific binding of the synprint peptide from $\alpha_{1A(rbA)}$ to syntaxin could be detected with immunoblot analysis at Ca^{2+} concentrations from 0 to 200 μM. As positive controls, binding of the synprint peptide of $\alpha_{1A(rbA)}$ to SNAP-25 was observed under similar conditions (Fig. 2), and the sharp, biphasic Ca^{2+} dependence of binding of α_{1B} to syntaxin was observed in parallel samples (Fig. 2).

In the next series of experiments, we tested whether the binding of the BI isoform of α_{1A} to syntaxin or SNAP-25 would show similar Ca^{2+} dependence to the binding of α_{1B} to those proteins (24). In binding experiments at Ca^{2+} concentrations from 0 to 200 μM, α_{1B} binding was Ca^{2+}-dependent with peak binding occurring at 10–20 μM for syntaxin (Fig. 3*A*) or SNAP-25 (Fig. 3*B*). In contrast, the BI isoform of α_{1A} did not show a difference in binding to syntaxin (Fig. 3*A*) or SNAP-25 (Fig. 3*B*) over a range of Ca^{2+} concentrations from 0 to 200 μM. Similarly, binding of $\alpha_{1A(rbA)}$ to SNAP-25 was not Ca^{2+}-

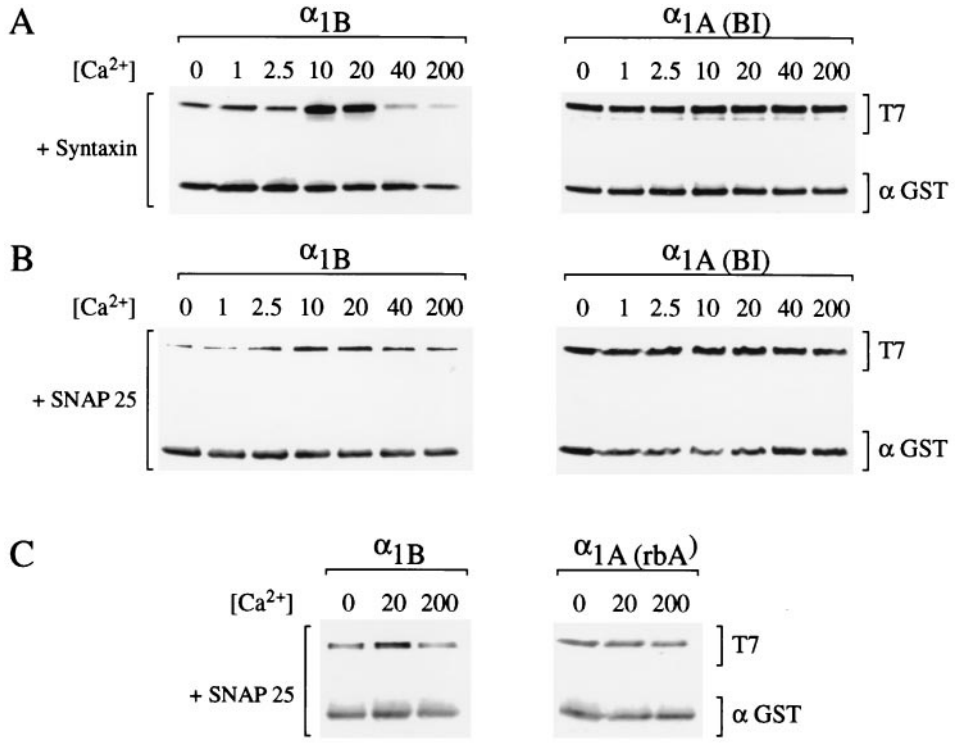

FIG. 3. Ca^{2+} dependence of binding of α_{1B} and the isoforms of α_{1A} to syntaxin and SNAP-25. Binding of the synprint peptides from α_{1B}, $\alpha_{1A(BI)}$, and $\alpha_{1A(rbA)}$ to immobilized GST-syntaxin or GST-SNAP-25 was measured as described in *Experimental Procedures* and the legend to Fig. 1 at the indicated concentrations of Ca^{2+}. Concentrations of GST fusion proteins were visualized with anti-GST antibody after stripping the membrane with SDS/2-mercaptoethanol. In these experiments no binding of the BI isoform of α_{1A} or of α_{1B} to GST was detected (data not shown).

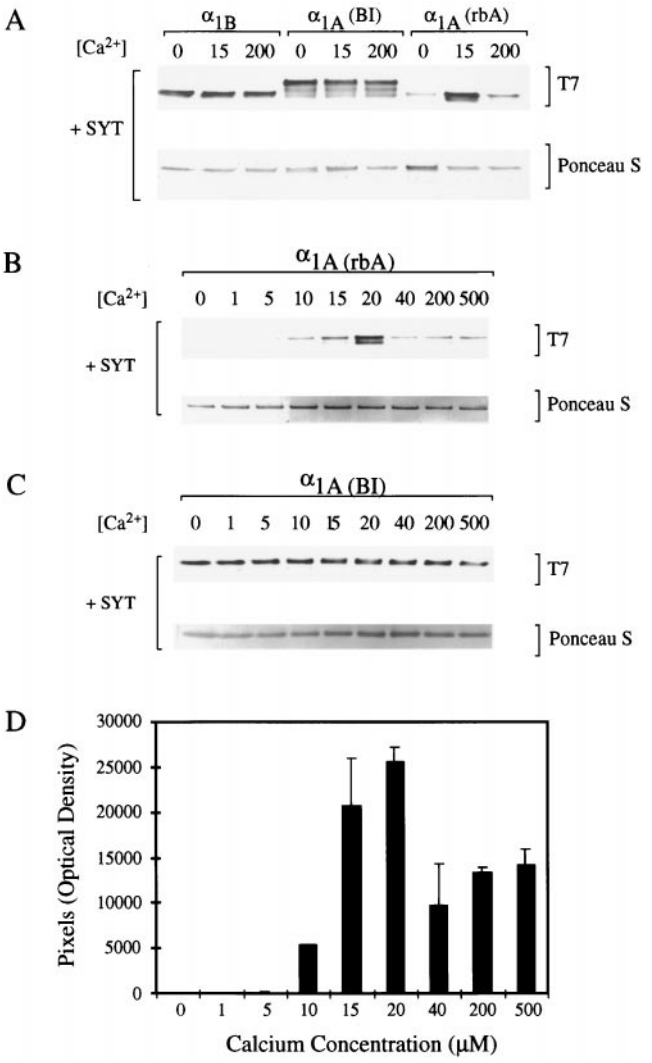

FIG. 4. Ca^{2+} dependence of binding of synprint peptides to synaptotagmin. (*A–C*) Binding of the synprint peptides from α_{1B}, $\alpha_{1A(BI)}$, and $\alpha_{1A(rbA)}$ to immobilized GST-synaptotagmin was measured as described in *Experimental Procedures* and the legend to Fig. 1 at the indicated concentrations of Ca^{2+}. Concentrations of GST proteins were visualized by staining with Ponceau S. (*D*) Results of three experiments like the one in *B* were quantitated by densitometry, averaged, and plotted as mean ± SEM vs. Ca^{2+} concentration.

dependent (Fig. 3*C*). These experiments demonstrate a marked difference in Ca^{2+}-dependent binding of SNARE proteins among the synprint peptides of α_{1B} and the rbA and BI isoforms of α_{1A}.

The Ca^{2+} dependence of α_{1A} and α_{1B} binding to synaptotagmin was tested by using similar methods (Fig. 4). Whereas the binding of α_{1B} and the BI isoform of α_{1A} to synaptotagmin was not Ca^{2+}-dependent, the binding of the rbA isoform of α_{1A} was strikingly Ca^{2+}-dependent (Fig. 4*A*). Binding of $\alpha_{1A(rbA)}$ to synaptotagmin was maximal at 15 μM with much reduced binding at 0 μM Ca^{2+} and 200 μM Ca^{2+}. This striking modulation of the level of binding of the rbA isoform of α_{1A} to synaptotagmin was examined over a wider range of Ca^{2+} concentrations (Fig. 4 *B* and *C*). Whereas the BI isoform of α_{1A} showed little variation in the level of binding to synaptotagmin over a range of Ca^{2+} concentration from 0 to 500 μM (Fig. 4*C*), binding of synaptotagmin to the rbA isoform of α_{1A} increased from a minimal level in the absence of Ca^{2+} to maximal binding with 15–20 μM Ca^{2+} and declined at Ca^{2+} concentrations above 20 μM (Fig. 4 *B* and *D*). The biphasic pattern of modulation of binding of synaptotagmin to $\alpha_{1A(rbA)}$ by Ca^{2+}

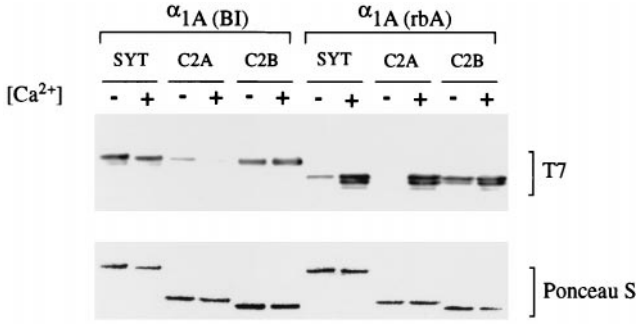

FIG. 5. Binding of the synprint peptides from the rbA and BI isoforms of α_{1A} to the C2 domains of synaptotagmin. Binding of the synprint peptides from α_{1B}, $\alpha_{1A(BI)}$, and $\alpha_{1A(rbA)}$ to 50 pmol of immobilized GST-synaptotagmin, GST-syt-C2A, and GST-syt-C2B was measured as described in *Experimental Procedures* and the legend to Fig. 1 at the indicated concentrations of Ca^{2+}. Concentrations of GST proteins were visualized by staining with Ponceau S. Ca^{2+} was present at a concentration of 15 μM where indicated.

is similar to that of binding of syntaxin or SNAP-25 to α_{1B} (Fig. 3; ref. 24), although binding of synaptotagmin to $\alpha_{1A(rbA)}$ is not inhibited as strongly by high Ca^{2+} concentration.

Binding of the Synprint Region of the BI and rbA Isoforms of α_{1A} to the C2 Domains of Synaptotagmin. Previous results showed that binding of the synprint region of α_{1B} to synaptotagmin was mediated through specific interaction with its C2B domain, with comparatively little binding to the C2A domain (26). As shown in Fig. 5, the BI isoform of α_{1A} also showed a specific interaction with the C2B domain of synaptotagmin with little binding to the C2A domain, as previously observed for α_{1B}. In contrast, the synprint peptide from the rbA isoform of α_{1A} bound strongly to the C2A domain of synaptotagmin as well as to the C2B domain (Fig. 5). Moreover, only the interaction with the C2A domain showed a striking dependence on Ca^{2+} concentration with markedly increased binding at 15 μM Ca^{2+} (Fig. 5). Thus, the Ca^{2+} dependence of interaction with the C2A domain of synaptotagmin is likely to be responsible for the Ca^{2+} dependence of the interaction of $\alpha_{1A(rbA)}$ with intact synaptotagmin (Fig. 4).

DISCUSSION

Specificity of Interaction of Synprint Peptides with Synaptic Proteins. This report provides further evidence for the specificity of the interactions of the Ca^{2+} channel synprint peptides with synaptic proteins, as summarized in Table 1. The synprint peptide from α_{1B} binds with high affinity to syntaxin and SNAP-25 in a Ca^{2+}-dependent manner with maximum binding at 10–20 μM, and it binds to synaptotagmin in a Ca^{2+}-independent manner through its C2B domain. The synprint peptide from the rbA isoform of α_{1A} binds to SNAP-25 in a Ca^{2+}-independent manner but does not bind to syntaxin *in vitro*. In contrast, it binds to synaptotagmin in a Ca^{2+}-dependent manner with maximum binding at 10–20 μM Ca^{2+}. Finally, the synprint peptide from the BI isoform of α_{1A} binds to syntaxin, SNAP-25, and synaptotagmin, but its binding is Ca^{2+}-independent in each case. It is surprising that the detailed patterns of binding specificity and

Table 1. Summary of the interactions of Ca^{2+} channel synprint peptides with synaptic proteins

	Syntaxin		SNAP-25		Synaptotagmin	
	Binding	Ca^{2+} dep	Binding	Ca^{2+} dep	Binding	Ca^{2+} dep
α_{1B}	+	+	+	+	+	−
$\alpha_{1A(rbA)}$	−	−	+	−	+	+
$\alpha_{1A(BI)}$	+	−	+	−	+	−

Ca^{2+} dep, Ca^{2+} dependence.

Ca^{2+} dependence of these three synprint peptides are different from each other. These results predict different interactions between these presynaptic Ca^{2+} channels and the transmitter release machinery in nerve terminals. Expression of these different Ca^{2+} channel subtypes in nerve terminals may confer specific functional and regulatory properties on the process of neurotransmitter release.

The similarity of the Ca^{2+} concentrations (10–20 μM) that induce maximum binding affinity of α_{1B} for syntaxin and SNAP-25 and of $\alpha_{1A(rbA)}$ for synaptotagmin suggests a similar mechanism by which Ca^{2+} binds to the synprint peptides and affects binding of SNARE proteins. As suggested previously (24), the optimal Ca^{2+} concentration may induce a conformational change that stabilizes low-affinity interactions of the synprint peptide with specific binding partners. Alternatively, a similar Ca^{2+} binding mechanism in each of the three presynaptic proteins may be responsible for the Ca^{2+} dependence of interaction with Ca^{2+} channels.

Differential Ca^{2+} Dependent Binding of Synaptotagmin to the Isoforms of α_{1A}. Our results show that the BI and rbA isoforms of α_{1A} both form specific interactions with synaptotagmin. The interaction of the rbA isoform of α_{1A} with synaptotagmin is dramatically affected by Ca^{2+}. In contrast, the interaction between the BI isoform of α_{1A} and synaptotagmin did not show a dependence on Ca^{2+} concentration, consistent with a recent report (27) and with the properties of the synprint peptide from α_{1B} (26). Evidently, the synprint regions of α_{1B} and $\alpha_{1A(BI)}$ interact similarly with synaptotagmin and Ca^{2+} whereas $\alpha_{1A(rbA)}$ interacts differently.

The Ca^{2+} dependence of binding of the synprint peptides to synaptotagmin is paralleled by their specificity of interaction with the C2 domains of synaptotagmin. Thus, the synprint peptides of α_{1B} and $\alpha_{1A(BI)}$ bind specifically to the C2B domain of synaptotagmin. In contrast, the synprint peptide of $\alpha_{1A(rbA)}$ binds to both the C2A and C2B domains of synaptotagmin, but only binding to the C2A domain is strongly Ca^{2+}-dependent, with maximum binding at 10–20 μM. The different binding specificities of these synprint regions for the C2A and C2B domains of synaptotagmin may reflect different interactions in the pathway to transmitter release initiated by Ca^{2+} influx through these different channel types.

Specific Localization and Function of the Isoforms of Presynaptic Ca^{2+} Channels. The α_{1B}, $\alpha_{1A(rbA)}$, and $\alpha_{1A(BI)}$ isoforms of presynaptic Ca^{2+} channels are differentially localized in synapses in the central nervous system (7, 8, 35). The fundamental mechanism of neurotransmitter release is thought to be the same at all fast synapses. Therefore, it is likely that the differences in interactions between Ca^{2+} channel subtypes and presynaptic SNARE proteins described here reflect subtle variations on a constant theme of transmitter release. We hypothesize that interactions between these presynaptic Ca^{2+} channels and the SNARE proteins are important to position docked vesicles near Ca^{2+} channels for efficient release in each case. However, the affinity of these interactions and their regulation by Ca^{2+} are specific for each Ca^{2+} channel type. The differences in interactions between Ca^{2+} channels and SNARE proteins may contribute to differences in the efficacy of initiation of transmitter release for Ca^{2+} influx through these different channels and may confer specialized regulatory properties that contribute to synaptic plasticity.

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