Modifications in the C Terminus of the Synaptosome-associated Protein of 25 kDa (SNAP-25) and in the Complementary Region of Synaptobrevin Affect the Final Steps of Exocytosis*

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Anabel Gil‡§, Luis M. Gutiérrez‡, Carmen Carrasco-Serrano‡, M. Teresa Alonso†, Salvador Viniegra, and Manuel Criado**

From the Department of Biochemistry and Molecular Biology and the Instituto de Neurociencias, Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas, 03550 San Juan, Alicante, Spain

Fusion proteins made of green fluorescent protein coupled to SNAP-25 or synaptobrevin were overexpressed in bovine chromaffin cells in order to study the role of critical protein domains in exocytosis. Point mutations in the C-terminal domain of SNAP-25 (K201E and L203E) produced a marked inhibition of secretion, whereas single (Q174K, Q53K) and double mutants (Q174K/Q53K) of amino acids from the so-called zero layer only produced a moderate alteration in secretion. The importance of the SNAP-25 C-terminal domain in exocytosis was also confirmed by the similar effect of mutations in analogous residues of synaptobrevin (A82D, L84E). The effects on the initial rate and magnitude of secretion correlated with the alteration of single vesicle fusion kinetics since the amperometric spikes from cells expressing SNAP-25 L203E and K201E and synaptobrevin A82D and L84E mutants had lower amplitudes and larger half-width values than the ones from controls, suggesting slower neurotransmitter release kinetics than that found in cells expressing the wild-type proteins or zero layer mutants of SNAP-25. We conclude that a small domain of the SNAP-25 C terminus and its counterpart in synaptobrevin play an essential role in the final membrane fusion step of exocytosis.

The SNAP1 (soluble NSF attachment protein) receptor (SNARE) hypothesis (1) has been crucial to our understanding of the molecular machinery responsible for exocytosis. The molecular events taking place during the exocytotic fusion of cellular and vesicular membranes should provide fundamental information about the mechanism of membrane fusion common to different membrane trafficking processes (2). The assembly of a ternary complex formed by the plasma membrane proteins syntaxin (3) and SNAP-25 (4) and the vesicle-associated protein synaptobrevin (5) is considered to be one of the molecular events driving vesicle priming, involving maturation steps needed to promote the apposition and final fusion of membranes (6). A heptad repeat structural motif typical of coiled-coils forming proteins is present and could be the basis for the formation of the core complex. This was confirmed by the observed increase in a-helix content following assembly of the complex (7, 8). In recent years, precise structural studies on the nature of the complex (9, 10) have shown that single fragments of 60–70 residues of syntaxin and synaptobrevin together with two segments of SNAP-25 form helices in a four-stranded coil. Although most of the interactions between these helices are hydrophobic, there is a polar layer embedded in the middle of this rod-shaped structure formed by three glutamines and one arginine (zero layer), which is believed to be critical for SNARE complex formation. In addition, functional studies using specific neurotoxins (11), antibodies against critical domains (12), peptides imitating regions of SNAREs (13), and overexpression of altered forms of these proteins (14, 15) have revealed important details about the participation of SNAREs in the exocytotic process. These and other studies suggest that SNARE interaction results in a tighter structure reducing the repulsive energy barrier between vesicular and plasma membranes. Assembly of the complex may proceed from the distal N-terminal domains of SNAREs assembled in parallel and, in “zipper-like” fashion, end with the interaction of C-terminal domains relatively close to the fusion pore. Even though there is not direct evidence for this model, a number of authors suggest that SNAREs assembly is directly linked to membrane fusion (16, 17).

We have overexpressed several mutants of SNAP-25 and synaptobrevin critical domains in adrenal chromaffin cells and studied their effect on catecholamine secretion. Our results indicate that specific residues at the SNAP-25 C-terminal domain and the equivalent synaptobrevin domain influence vesicle fusion kinetics, suggesting a role in the very last events of the exocytotic process. By contrast, zero layer amino acid mutants, despite their ability to negatively affect the formation and stability of SNAREs complexes assembled in vitro, did not produce strong alterations.

EXPERIMENTAL PROCEDURES

Generation of Constructs of GFP Coupled to SNAP-25 or Synaptobre- 
vin—To produce an in-frame fusion of SNAP-25 to the C terminus of GFP, the cDNA corresponding to the SNAP-25a isoform (18) was cloned into the expression vector pEGFP-C3 (CLONTECH, Palo Alto, CA) as previously described (14). The generation of the nine amino acids C-terminal deletion (Δ9) and the point mutation L203E has also been
described (14). The strategy for generating the other C-terminal mutants was based in the presence of a TsA site at the 5’-end of the region containing the nucleotides to be mutated and a BamHI site at the 3’-end. Complementary oligonucleotides carrying the desired mutations and the mentioned restriction sites were annealed and cloned into the corresponding sites in place of the GFP coding region. The point mutation Q53K was generated by PCR of a fragment corresponding to the N terminus of SNAP-25 using the GFP-SNAP-25 construct as template, a sense primer (5’-GGCGATGACGACCTGTAC-3’-sense) and an antisense primer, which included the sequence to be mutated (indicated in uppercase) 5’-CCGACGATCCGATGTCGTCATCAG-3’ (antisense) corresponding to the C terminus of SNAP-25 and close to the junction between GFP and SNAP-25 and an antisense primer containing the sequence to be mutated (5’-AAATGGTAGTGATCTTCATGTTCTCCTTCATGTTCAAC-3’). The PCR product was digested with XhoI and PvuI and used to replace the equivalent fragment in the GFP-SNAP-25 construct, which had been digested with the same enzymes. The point mutation Q174K was generated by PCR of a fragment corresponding to the C terminus of SNAP-25 with a sense primer containing the sequence to be mutated (5’-CAATGGATGATG-3’-sense) and an antisense primer downstream of the C terminus of SNAP-25 (5’-CTACGATGGGTTTCTCGTTCATT-3’). The amplified DNA carried an internal ClaI site, close to the 5’-end, and a BamHI site at the 3’-end. These enzymes were used to substitute the original SNAP-25 sequence by the modified one in the GFP-SNAP-25 construct. The double mutant Q53K/Q174K was then obtained by combining the single ones through enzyme restriction and ligation reactions.

To produce an in-frame fusion of synaptobrevin to the N terminus of GFP, the coding region corresponding to synaptobrevin II (19) was amplified by PCR with the following primers: 5’-GCCGATTTCGGCGCATGTCGCAC-3’ (sense) and 5’-GCCGATGCACGCCATGTCGTC-3’ (antisense). The PCR product was digested with EcoRI and BamHI and cloned into the same sites of the expression vector pEGFP-N1 (CLONTECH, Palo Alto, CA). The strategy for generating the synaptobrevin mutants (A81D, A82D and L84E) was based on PCR amplifications. The corresponding products were modified one in the GFP-SNAP-25 construct. The double mutant Q53K/322K was then obtained by combining the single ones through enzyme restriction and ligation reactions.

Confocal Microscopy Studies of the Distribution of GFP-Synaptobrevin Constructs—Briefly, cells overexpressing GFP-synaptobrevin constructs were fixed using a 4% paraformaldehyde in phosphate-buffered saline solution during 20 min. Then cells were washed with phosphate-buffered saline solution and mounted in 80% glycerol in the same solution. Fluorescence was investigated using a Laser Scanning confocal TCS Leika microscope. Usually, eight confocal layers covering the total cell volume were obtained (1.25-μm thickness (z)), and individual layers or projections were used to study fluorescence distribution.

Amperometric Measurements—Amperometric measurements were carried out as previously described (21). Efficiency of virus infection was determined for the different mutants. All data was expressed as mean ± S.E. from experiments performed in a number (n) of individual cells. Data represent experiments performed with cells from at least three different cultures.

RESULTS

GFP-SNAP-25 Constructs Are Heavily Expressed and Functional in Chromaffin Cells Infected with Herpes Simplex Modified Virus (Amplcides)—Overexpression of native and mutant...
forms of SNAP-25 in chromaffin cells is a valuable tool when studying its role in neurosecretion (14, 15). In initial studies we used calcium phosphate and electroporation transfection methods, resulting in low levels of transfection (less than 1%). We therefore decided to use amplicons, modified HSV (27). Viral infection produced a greater proportion (15–30%) of cells expressing high amounts of GFP-SNAP-25 constructs, as indicated by their brilliant green fluorescence. We used single cell amperometry (11 micron carbon fiber electrodes) to test the ability of these cells to secrete catecholamines in response to a depolarizing stimulus. Upon stimulation, characteristic spikes depicting single fusion events were obtained for a number of individual cells (Fig. 1A). The secretory profile was obtained by the integration and averaging of amperograms from 22 to 60 individual cells (Fig. 1C). HSV-infected cells incorporating the GFP-SNAP-25 construct and cells expressing GFP alone (Fig. 1A and B) produced about 50% of the secretory activity normally found in uninfected cells (not shown). The close match of GFP and GFP-SNAP-25 secretory rates when normalized, indicates that secretory kinetics were unaffected by expression of the GFP-SNAP-25 construct and that the reduction in the number of released vesicles observed in infected cells was due to the viral infection itself rather than to a possible inhibition of the secretory machinery by overexpression of SNAP-25. Validity of the HSV amplicon system for testing SNAP-25 function was further confirmed by the catecholamine release in cells overexpressing a GFP-SNAP-25 construct incorporating the mutation of leucine 203 to glutamic acid (L203E) that had previously been shown to greatly affect secretion kinetics (14). The expression of this construct partially abrogated the overall extent of the secretory response (Fig. 1A shows a 70% reduction in the sustained response compared with the control GFP-SNAP-25 construct), and initial rate of release (Fig. 1B, about 0.2 vesicles/s, 32% of the rate found for the GFP-SNAP-25 construct). These results are consistent with those obtained by conventional transfection methods (14). Similar results (Fig. 1B) were observed using a construct lacking the last nine C-terminal residues of this SNARE (GFP-SNAP-25 Δ9) and therefore equivalent to the proteolyzed form produced by the action of botulinum toxin A (28, 29). These results indicated that the amino acid L203, which is absent in this construct as well as in the SNAP-25 polypeptide cleaved by botulinum neurotoxin A, is critical for exocytosis.

The amplicon system was further used to examine the secretory response of cells expressing SNAP-25 proteins mutated at amino acids located in critical areas. It has been suggested that amino acid residues in the zero layer (Gln-53 and Gln-174 of SNAP-25) are critical for SNARE complex formation (9, 30). The single mutation in Q174K showed a relatively small effect on secretion when compared with the L203E mutant (both magnitude and initial rate of catecholamine release were reduced 35% in comparison to control values, see Fig. 1, A and B). A slight effect was also observed with the Q53K mutation (also present in the zero layer but forming part of the SNAP-25 N-terminal domain), which released 15 ± 2 vesicles (n = 20) during prolonged depolarization (52% inhibition, data not shown). Double mutations in the previously mentioned glutaminies (Q53K/Q174K) produced lower inhibition (50% in extent and initial rate of secretion, Fig. 1B) than that obtained with L203E. Although these data imply a role for the zero layer in secretion, it appears to be less important than the part played by Leu-203 at the SNAP-25 C terminus.

**Mutational Study of Other Amino Acids Near Leu-203**—To assess the importance of regions flanking Leu-203 in molecular events leading to membrane fusion we studied the possible role of residues at the SNAP-25 C-terminal domain in this process. To this end we mutated Ala-199 and Met-202, which have been, respectively, assigned to the seventh and eighth hydrophobic layers of the four-helix bundle in the synaptic fusion complex (30), as well as Lys-201, which is located in between these two. The secretory behavior of cells expressing mutants A199E and M202E was slightly affected with only the initial rate of vesicle release being slower in the M202E mutant (Fig. 2). Mutant K201E had a greater inhibitory effect on the total number of vesicles secreted during cell depolarization, and the initial rate of vesicle release was also retarded (Fig. 2). These results suggest that hydrophobic amino acids in the C-terminal domain of SNAP-25, such as Leu-203 and to a lesser extent Met-202, play a relevant role in membrane fusion events leading to exocytosis and that electrostatic interactions through residue Lys-201 could also be involved in this process.

**Mutations in Synaptobrevin Residues Close to the SNAP-25 C-terminal Domain Affect Exocytosis**—Current structural data
suggest that several hydrophobic synaptobrevin amino acids such as Ala-81, Ala-82, and Leu-84 could interact with Leu-203 of SNAP-25. We therefore decided to mutate these synaptobrevin residues in GFP constructs linked to the synaptobrevin II C-terminal domain, which were also overexpressed following ampiclon infection of bovine chromaffin cells (Fig. 3). Constructs with wild-type synaptobrevin (Fig. 3A) as well as the A81D (Fig. 3B), A82D (Fig. 3C), and L84E (Fig. 3D) mutants, were expressed at similar levels in a punctate pattern suggesting their location in chromaffin vesicles. The secretory activity of cells expressing these constructs was studied using depolarizing stimuli. Cells expressing wild-type synaptobrevin coupled to GFP (SV WT) had an exocytotic behavior resembling that observed in cells expressing GFP and the GFP construct coupled to wild-type SNAP-25 (Fig. 4) in that neither the kinetics nor the level of secretion obtained after 1 min of continuous depolarization was modified. Expression of mutant A81D had a relatively low impact on the release of catecholamines, which showed a 35% inhibition in both overall extent and initial rate of release (Fig. 4). Mutants A82D and L84E had a more pronounced effect on secretory behavior, reaching levels close to 70% inhibition (Fig. 4) similar to that observed in the mutant L203E of SNAP-25. These data strengthen the notion that the vesicle fusions analyzed were produced in the electrode proximity. Amplitudes were also similar in cells expressing GFP-SNAP-25 or GFP-synaptobrevin were characterized by very similar distributions, showing means taken from averaged individual cell amplitudes of 70, 59, and 50 pA, respectively (Table I). Amplitudes were also similar in cells expressing single (Q174K, Fig. 5D and Table I; Q53K, Table I) and double (Q174K/Q53K, Table I) mutants of the zero layer as well as A199E and M202E mutants of the SNAP-25 C terminus (429 events from 32 cells; G, H, and I). Analysis included spikes with amplitude larger than 5 pA and half-width shorter than 75 ms to ensure that the vesicle fusions analyzed were produced in the electrode proximity. A, D, G, and J, represent amplitude distributions. Amplitude was measured and data binned at 10 pA intervals. B, E, H, and K show single fusion charge distributions. Event charge was calculated by trapezoidal integration and histograms of Q\textsuperscript{1/3} were built using 0.1 pC\textsuperscript{1/3} bin size. C, F, I, and L, represent spike half-width time distributions. Half-width was measured by subtraction of the half-height times corresponding to the rising and falling portions of each spike. Data were binned at 2.5 ms intervals.

FIG. 4. Depolarization-evoked secretion in chromaffin cells overexpressing different GFP-synaptobrevin constructs. Secretion was monitored by amperometry as described in previous figures. After building of event cumulative responses for individual cells, the average secretion was obtained from control GFP-synaptobrevin wild-type; SV WT, (n = 42 cells), SV A81D (n = 26), SV A82D (n = 27), and SV L84E (n = 26).

FIG. 5. Effect of the expression of SNAP-25 and synaptobrevin mutants on single event characteristics. Individual spikes were analyzed from depolarization responses of GFP-SNAP-25 full-length (903 events from 23 cells; A, B, and C), Q174K mutant of the zero layer (481 events from 36 cells; D, E, and F), and L203E mutant of the SNAP-25 C terminus (429 events from 32 cells; G, H, and I). Analysis included spikes with amplitude larger than 5 pA and half-width shorter than 75 ms to ensure that the vesicle fusions analyzed were produced in the electrode proximity. A, D, G, and J, represent amplitude distributions. Amplitude was measured and data binned at 10 pA intervals. B, E, H, and K show single fusion charge distributions. Event charge was calculated by trapezoidal integration and histograms of Q\textsuperscript{1/3} were built using 0.1 pC\textsuperscript{1/3} bin size. C, F, I, and L, represent spike half-width time distributions. Half-width was measured by subtraction of the half-height times corresponding to the rising and falling portions of each spike. Data were binned at 2.5 ms intervals.
tudes over 75 pA, which were relatively abundant in control cells were less frequent in these cases. These results are in good agreement with those reported for C-terminal deletions in transfected chromaffin cells (14). On the other hand, the alteration seen in mean amplitude was not associated with a change in the charge released per event, which remained relatively unaltered in control, zero layer, and C terminus mutants with average values ranging from 1.3 to 0.94 pC\(^{1/3}\) (Fig. 5, B, C, and E and Table I). By contrast, statistically significant differences in the half-height width distribution (half-width) were observed for L203E, K201E and A9 mutants (p < 0.002, analysis of variance test) when compared with values obtained for wild-type SNAP-25 (see Fig. 5, C, F, and I and Table I). The half-width distributions of wild-type SNAP-25 as well as mutants Q174K, Q53K, and A199E had a high proportion of short \(t_{1/2}\) value events (ranging from 6 to 14 ms and mean values around 18–22 ms), whereas cells expressing L203E, K201E, and A9 mutants had longer and smaller events (24–26 ms mean values), the presence of narrow spikes corresponding to vesicles fusing with short half-width values being less frequent. The amperometric events from synaptobrevin constructs were also analyzed. In this case cells expressing mutant A82D were characterized by lower average amplitude (Fig. 5J) and higher half-width (Fig. 5L) values (p < 0.001, Table I). The differences with respect to the controls were even greater than those observed with the SNAP-25 mutants. The effect was less pronounced with the L84E mutant (p < 0.05, Table I), despite a marked decrease in overall secretion (see Fig. 4). The A81D mutant showed no difference from the wild-type construct (Table I).

In summary, only certain amino acids of SNAP-25 and synaptobrevin, presumably in close proximity each other, appear to play a central role in the membrane fusion event immediately preceding the release of stored catecholamines.

**Thermostability of SNARE Complexes Formed by Different Mutants in Vitro Does Not Fully Correlate with Their Effects on the Exocytotic Process**—The previously described forms of SNAP-25 and synaptobrevin together with syntaxin were used to study the in vitro stability of their ternary complexes in an attempt to understand the molecular basis for the alterations observed in exocytosis. Initially, complexes were formed using

The values are expressed as the mean ± S.E. from experiments performed in a number (n) of cells. In the table SNAP-25 and SV represent averaged control and post-control values.

| Experimental condition | Amplitude (pA) | Integrated current (pC\(^{1/3}\)) | Half-width (ms) |
|------------------------|---------------|----------------------------------|-----------------|
| Control cells (n = 23)  | 70 ± 8        | 1.05 ± 0.03                      | 21 ± 1          |
| SNAP-25 (n = 45)       | 59 ± 4        | 1.01 ± 0.02                      | 19 ± 1          |
| SNAP-25 (Q174K, n = 36)| 59 ± 6        | 0.99 ± 0.02                      | 20 ± 2          |
| SNAP-25 (Q53K, n = 66)| 63 ± 4        | 1.06 ± 0.02                      | 22 ± 1          |
| SNAP-25 (Q174K/Q53K, n = 36)| 61 ± 4| 1.38 ± 0.3                    | 21 ± 1          |
| SNAP-25 (A199E, n = 38)| 77 ± 8        | 1.02 ± 0.05                      | 18 ± 1          |
| SNAP-25 (K201E, n = 66)| 42 ± 5        | 0.94 ± 0.02                      | 24 ± 1          |
| SNAP-25 (M202E, n = 21)| 65 ± 7        | 1.09 ± 0.03                      | 20 ± 1          |
| SNAP-25 (L203E, n = 32)| 34 ± 3        | 0.97 ± 0.03                      | 26 ± 2          |
| SNAP-25 (A9, n = 26)   | 49 ± 4        | 1.02 ± 0.03                      | 25 ± 1          |
| SV (n = 42)            | 50 ± 6        | 0.99 ± 0.03                      | 21 ± 1          |
| SV (A81D, n = 26)      | 51 ± 5        | 0.96 ± 0.02                      | 21 ± 2          |
| SV (A82D, n = 27)      | 29 ± 3        | 0.94 ± 0.02                      | 28 ± 2          |
| SV (L84E, n = 26)      | 47 ± 5        | 1.04 ± 0.04                      | 24 ± 2          |

* p < 0.05.
** p < 0.002.
*** p < 0.001 using the analyses of variance test compared with the respective control values (SNAP-25 and SV control and post-control conditions).

**Fig. 6.** Thermal stability of ternary complexes formed by altered forms of SNAP-25. Synaptobrevin 2B, syntaxin 1A, and the different mutants of SNAP-25 were expressed and purified as GST fusion proteins as described under “Experimental Procedures.” After formation of the SDS-resistant complexes during 1 h at room temperature, these were heated during 3 min and then complex dissociation was stopped by cooling the samples to 4 °C. A, examples of ternary complex thermostability corresponding to full-length SNAP-25 (SNAP-25), and to different mutants (SNAP-25 A9, L203E and Q174K). B, thermostability curves obtained in the conditions described in A for data corresponding to three experiments. Results were expressed as percentage of the maximum amount of formed complex and given as means ± S.E.
ferences found in their individual fusion kinetics parameters, the thermostability of the ternary complexes formed by these synaptobrevin mutants is very similar.

**DISCUSSION**

The use of botulinum neurotoxin has been crucial to understand the key role played by the SNAP-25 C terminus in the secretory process of neuroendocrine chromaffin and PC12 cells (22, 32–35). More recently, the use of constructs containing modifications in this domain (14) has facilitated access to protein segments different from those defined by toxin cleavage. Thus, the substitution of endogenous SNAP-25 by an overexpressed mutant in which the last six C-terminal amino acids are deleted, alters the single vesicle fusion kinetics analyzed by amperometry (36), suggesting the involvement of these residues in the very last events of granule exocytosis. The data presented here expands on our previous findings and provides new insights into the exocytic role of single amino acids from the highly conserved layers of interacting amino acids in the four-helix bundle play a fundamental role in the last membrane fusion steps of exocytosis and that the zero layer may participate in up-stream stages of this process. These conclusions are supported by a recent piece of work (15) in which the effects of the Δ9 deletion and Q174L mutation were studied by combining flash photolysis and capacitance techniques. It was deduced that the zero layer is critical for the formation of SNARE complexes but, in contrast to the last nine C-terminal amino acids, plays no role in the dynamic equilibrium between the two exocytic burst components. The significance of Leu-203 and Lys-201 at the SNAP-25 C terminus was further stressed when mutations in synaptobrevin residues such as Ala-82 and Leu-84, which have been mapped in their immediate proximity, (9, 10, and the corresponding entries of the Protein Data Bank 1SFC, 2B0U), produced a very similar impact on both overall secretory properties (Fig. 4) and the individual vesicle fusion characteristics (Table I).

As expected from their marked effect on secretory cell behavior, complex thermostability decreased in the presence of C terminus-altered forms of SNAP-25 such as Δ9 and L203E as well as in their functional synaptobrevin counterparts A82D and L84E. However, Q174K and Q53K mutants of the SNAP-25 zero layer, which did not affect secretion in a drastic way, had a strong effect on the in vitro formation and stability of the ternary complex. As such there is not a complete correlation between the functional effects of the mutants in vivo and their ability to modify the properties of the ternary complex in vitro. In this sense, the most extreme case was the double mutation in the zero layer arginines contributed by SNAP-25, which had a severe impact on the complex since we could not detect its formation at room temperature but which was able to interact in vitro since it showed an intermediate behavior between that of the controls and the most inhibitory C-terminal mutants. These data point to the fact that observations on the formation and stability of the synaptic fusion complex in vitro should be interpreted with caution and validated with functional data.

What could be the role of SNAP-25, Leu-203, and Lys-201...
and synaptobrevin Ala-82 and Leu-84 amino acids during the final membrane fusion steps of exocytosis? As previously indicated, these amino acids had not been assigned to any of the conserved layers of amino acids whose side chains contribute to synaptic fusion complex formation. Nevertheless, the hydrophobic nature of SNAP-25 Leu-3 and synaptobrevin Ala-82 and Leu-84 suggests their involvement in leucine zipper mechanisms stabilizing protein-protein interactions. In addition, the importance of these hydrophobic residues may reside in their location within the SNAP-25 C terminus and the complementary region of synaptobrevin where they could: (a) reinforce the structural integrity of the zipper movement toward the C-terminal regions of the participating SNAREs (37), (b) help form a tight state of the complex after zipper and participate in its final closure, and (c) drive the membrane fusion by apposition of the membranes below a critical distance. Regarding Lys-201, and according to the actual crystallograpical models, it may be located so that its side chain is oriented toward the outside of the four-helix bundle. Its positively charged nature suggest an involvement in electrostatic interactions with other protein factors that may be present as components of the basic machinery responsible for vesicle docking, maturation, and at least part of the final membrane fusion mechanism. An alternative hypothesis would be that Leu-203 and Lys-201 SNAP-25 mutations and Ala-82 and Leu-84 synaptobrevin mutations disrupt the overall structure of the corresponding core complex domain. However, this disruption should be specific for these amino acids and not for others located in the same area, such as SNAP-25 Ala-199 and synaptobrevin Ala-81. In either case, the experiments described in this paper demonstrate an important participation by specific amino acids of the SNAP-25 C-terminal domain and the corresponding synaptobrevin domain in the final membrane fusion steps of the exocytic process.

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