SNAP-25 and its ubiquitous homolog SNAP-23 are members of the SNARE family of proteins that regulate membrane fusion during exocytosis. Although SNAP-23 has been shown to participate in a variety of intracellular transport processes, the structural domains of SNAP-23 that are required for its interaction with other SNAREs have not been determined. By employing deletion mutagenesis we found that deletion of the amino-terminal 18 amino acids of SNAP-23 (encoded in the first exon) dramatically inhibited binding of SNAP-23 to both the target SNARE syntaxin and the vesicle SNARE vesicle-associated membrane protein (VAMP). By contrast, deletion of the carboxyl-terminal 23 amino acids (encoded in the last exon) of SNAP-23 does not affect SNAP-23 binding to syntaxin but profoundly inhibits its binding to VAMP. To determine the functional relevance of the modular structure of SNAP-23, we overexpressed SNAP-23 in cells possessing the capacity to undergo regulated exocytosis. Expression of human SNAP-23 in a rat mast cell line significantly enhanced exocytosis, and this effect was not observed in transfectants expressing the carboxyl-terminal VAMP-binding mutant of SNAP-23. Despite considerable amino acid identity, we found that human SNAP-23 bound to SNAREs more efficiently than did rat SNAP-23. These data demonstrate that the introduction of a “better” SNARE binder into secretory cells augments exocytosis and defines the carboxyl terminus of SNAP-23 as an essential regulator of exocytosis in mast cells.

Characterization of the molecular mechanism of membrane-membrane fusion in living cells has revealed that the proteins regulating this process are conserved in systems such as protein secretion in yeast, synaptic vesicle exocytosis, and intracellular vesicle fusion during membrane traffic in mamalian cells (1, 2). One key set of proteins that regulate these diverse biological processes are called SNAREs. SNAP proteins are present on both vesicle membranes (vesicle SNAREs, or v-SNAREs) and on target membranes (target SNAREs, or t-SNAREs). The v-SNAREs include proteins of the VAMP family, whereas t-SNAREs include proteins of the syntaxin and SNAP-25 families. The neuronal SNAREs include syntaxin 1A, SNAP-23 and VAMP 2, and these proteins readily associate with one another to form a stable ternary complex that is essential for synaptic vesicle exocytosis.

The neuronal SNARE complex is composed of a parallel four-helical bundle of coiled-coils (3, 4). Syntaxin 1A and VAMP 2 (tethered to opposing membranes) each contain one coiled-coil domain, and SNAP-25 contains two coiled-coil domains, one at its amino terminus and one at its carboxyl terminus (5). By employing deletion analysis, several groups have analyzed the domains of the neuronal SNAREs that are essential for efficient SNARE complex assembly (5–7). Both coiled-coils of SNAP-25 are important for its interaction with VAMP 2 (5) while the major syntaxin binding region is present in the first coiled-coil (5, 6). In agreement with these data, proteolysis of SNAP-25 by Botulinum neurotoxins that remove the carboxy terminus of SNAP-25 does not alter its binding to syntaxin but dramatically inhibits its binding to VAMP 2 (6). Under conditions of neurotoxin poisoning exocytosis is abolished (8), highlighting the functional importance of the carboxy-terminal region of SNAP-25 to VAMP binding.

Two non-neuronal homologs of SNAP-25 have been identified, and they have been named SNAP-23 (Refs. 9–11) and SNAP-29 (Refs. 12 and 13). These isoforms exhibit a significant similarity at the protein level with SNAP-25, and each possesses the two coiled-coils necessary for the formation of SNARE complexes. The role of SNAP-29 is still unresolved, with one study suggesting that it plays a role primarily in intra-Golgi traffic (13) and another suggesting that SNAP-29 regulates a variety of intracellular traffic events (12). On the other hand, SNAP-23 is thought to play a role both in regulated and constitutive protein trafficking pathways in non-neuronal cells. For example, SNAP-23 is involved in diverse protein trafficking events such as GLUT4 trafficking in adipose cells (14, 15), compound exocytosis in mast cells (16), polarized protein traffic (17), platelet dense core granule release (18), and transferrin receptor recycling (19).

Because of its wide tissue distribution, its ability to interact with several syntaxin and VAMP isoforms, and the aforementioned functional data, SNAP-23 is likely to be a key player in many distinct protein trafficking events in non-neuronal cells. Both SNAP-23 and SNAP-25 are thought to function primarily as t-SNARE heterodimers together with syntaxin (20, 21); however, the ability of SNAP-23 to replace SNAP-25 in regulated exocytosis is limited (22). A systematic analysis of the domains of SNAP-23 that are required for its binding to syntaxin and VAMP could allow the identification of SNAP-23 mutants that could alter intracellular granule-plasma membrane fusion events in distinct cell types in vivo.

In this study, we have characterized the interaction of SNAP-23 with syntaxin 4 and VAMP 2. These SNARE isoforms were chosen because complexes containing these proteins with SNAP-23 are common in exocytic events in non-neuronal cells.
SNAP-23 in Mast Cell Exocytosis

(23, 24). Truncation analyses revealed that while the carboxy-terminal 23 amino acids of SNAP-23 are not required for SNAP-23 binding to syntaxin, they are essential for SNAP-23 binding to VAMP in vitro and in vivo. Overexpression of wild-type human SNAP-23 in rat mast cells resulted in enhanced exocytosis from the cells, and this effect was completely abolished in mast cells expressing a human SNAP-23 carboxy-terminal VAMP-binding mutant. Thus, these data demonstrate that SNAP-23 mutants that bind to syntaxin but not to VAMP are unable to support exocytosis from rat mast cells and point to the SNAP-23 carboxyl terminus as an important regulator of exocytosis.

EXPERIMENTAL PROCEDURES

Plasmids and Recombinant Proteins—cDNAs encoding full-length human SNAP-23 (9), mouse SNAP-25b (25), rat syntaxin 1A (21), and human syntaxin 4 (26) were subcloned into pcDNA3 (Invitrogen). FLAG-tagged rat VAMP 2 in pRC-CMV was the gift of Dr. Richard Scheller (Stanford University). GST-syntaxin and GST-VAMP fusion proteins were generated by standard cloning and expression strategies in Escherichia coli (9). The recombinant proteins were affinity-purified using glutathione-Sepharose beads according to the manufacturer's instructions (Amersham Pharmacia Biotech). Deletion mutants of SNAP-23 in pcDNA3 were generated by the polymerase chain reaction using mutant oligonucleotide primers encoding stop codons. The sequence of all mutants was verified by automated DNA sequence analysis.

In Vitro Binding Studies—Radiolabeled proteins were generated by in vitro translation reactions using rabbit reticulocyte lysate in the presence of [35S]methionine using the T7 Quick in vitro transcription/translation kit (Promega). For in vitro binding studies, GST, GST-syntaxin 4, or GST-VAMP 2 (10 μg each) were immobilized on glutathione-Sepharose beads and were mixed with in vitro translated wild-type or mutant proteins in a final volume of 200 μl of binding buffer (4 mM HEPES, 100 mM NaCl, 3.5 mM CaCl2, 3.5 mM MgCl2, 1 mM EDTA, 0.1% Nonidet P-40, pH 7.4) for 2 h at 4 °C. All incubations contained nearly identical amounts of in vitro translated material, and total amount of rabbit reticulocyte lysate in all tubes was kept constant by using aliquots from a mock translation mixture. After incubation, the beads were washed three times with 1 ml of washing buffer (50 mM Tris, 100 mM NaCl, 2.5 mM MgCl2, 0.1% Nonidet P-40, pH 8.0) and the resulting pellet was resuspended in SDS-PAGE sample buffer containing 0.1% β-mercaptoethanol. The samples were heated to 95 °C for 5 min, and the supernatant fractions were analyzed by SDS-PAGE (27).

The gels were stained with Coomassie Brilliant Blue R-250 to visualize protein bands and impregnated with Enlightening (PerkinElmer Life Sciences) for fluorography. The gels were dried and exposed to PhosphorImager screens, and the intensity of each band was quantitated by PhosphorImager analysis using a Molecular Dynamics model HFF PhosphorImager.

Antibodies, Immunoprecipitation, and Immunoblotting—Anti-peptide rabbit antisera recognizing the amino terminus of human SNAP-23 have been described previously (25). A broadly reactive SNAP-23 antiserum was generated by immunizing rabbits with purified recombinant human β-Hex-SNAP-23 fusion protein. To ensure that these antisera reacted equally well with SNAP-23 truncation mutants, we analyzed in vitro translated wild-type or mutant proteins by immunoprecipitation and immunoblot analyses. These studies confirmed that the introduction of these mutations did not alter the immunogenicity of the various constructs used in this study. The SNAP-25 monoclonal antibody was from Sternberger Monoclonals, Inc. (Baltimore, MD), the syntaxin 4 monoclonal antibody was from Transduction Labs, and the FLAG-epitope monoclonal antibody M5 was from Sigma.

Immunoblot analysis of cell lysates or immunoprecipitation from cell lysates was performed as previously described (27). For immunoprecipitations from cell extracts, cells were briefly washed with Hanks' balanced salt solution and lysed at 4 °C with 1 ml of lysis buffer (1% Triton X-100, 100 mM NaCl, 2.5 mM MgCl2, 0.1% Nonidet P-40, pH 7.5) containing 5 mM iodoacetamide. The lysates were subjected to centrifugation at 14,000 rpm at 4 °C to remove cell debris, and the cleared lysates were used for immunoprecipitation reactions. Immunoprecipitated proteins were analyzed on 10.5% SDS-PAGE gels and immunoblotting or fluorography (as indicated). In some cases the gels were stained with Coomassie Brilliant Blue R-250 to confirm that samples contained similar amounts of GST fusion proteins.

Transfection of HeLa and RBL Cells—Subconfluent HeLa cells were transfected using LipofectAmine (Life Technologies, Inc.) according to the manufacturer's specifications. Transfected HeLa cells were generated by incubating 106 cells in 10 ml of fresh medium 18–24 h after transfection. The rat mast cell line RBL-2H3 was maintained as adherent cultures in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO2 at 37 °C. RBL cells (5 × 106/ml) in Dulbecco's modified Eagle's medium without serum were transfected by electroporation (250 V, 960 microfarad) using 10 μg of empty vector, 10 μg of wild-type human SNAP-23 in pcDNA3, or 10 μg of human SNAP-23-(1–168) in pcDNA3. Dulbecco's modified Eagle's medium/fetal bovine serum and allowed to recover overnight. G418 (0.5 mg/ml) was added to the cultures, and stable transfectants were selected within 7 days by picking individual colonies with a sterile pipette tip. Stable transfectants were maintained in Dulbecco's modified Eagle's medium/fetal bovine serum containing 0.25 mg/ml G418.

Stimulation of RBL Cell Exocytosis—Exocytosis in the RBL-2H3 mast cells was triggered using phorbol 12-myristate 13-acetate and ionomycin. RBL cells (1 × 106) were washed with RPMI (without phenol red), and degradation was induced by adding phorbol 12-myristate 13-acetate (10 nM) and ionomycin (1 μM) in a final volume of 1.5 ml. Plates were incubated at 37 °C, aliquots of the medium were withdrawn at various times, and β-hexosaminidase activity released into the medium was measured. Mock degradation studies were carried out in parallel using vehicle in medium alone. At the end of the assay, cells were lysed with 1 ml of 1% Triton X-100 in RPMI to measure residual cell-associated β-hexosaminidase. To determine β-hexosaminidase activity, aliquots (20 μl) of the supernatants and cell lysates were incubated with 50 μl of the substrate solution (1.3 mg/ml of p-nitrophenyl-N-acetylβ-D-glucosaminide (Sigma) in 0.1 M citrate buffer (pH 4.5) for 90 min at 37 °C. The reaction was terminated by the addition of 100 μl of 0.2 M NaOH/0.2 M glycine. Absorbance was read at 405 nm in an enzyme-linked immunosorbent assay reader, and the amount of exocytosis was expressed as the percentage of total β-hexosaminidase activity present in cells.

RESULTS

Identification of SNAP-23/SNARE Interacting Domains in Vitro—SNAP-23 is known to be an important participant in the formation of the SNARE complex that leads to membrane-membrane fusion. In our attempt to identify the regions of SNAP-23 that are essential for its function, we set out to identify the domains of SNAP-23 that are essential for its ability to form binary SNARE complexes. We have recently cloned the entire SNAP-23 gene (28), and because distinct exons often encode functional domains in proteins, we generated a series of amino- and carboxyl-terminal truncation mutants of human SNAP-23 based on the exon structure of SNAP-23 (Fig. 1A). These mutants correspond to deletions of the first coding exon (SNAP-23-(19–211)), the first and second coding exons (SNAP-23-(33–211)), the first four coding exons (including the first coiled-coil domain, SNAP-23-(76–211)), the last coding exon (SNAP-23-(1–188)), and the last two coding exons (SNAP-23-(1–140)).

In vitro binding assays revealed that deletion of 18 amino acids from the amino terminus of SNAP-23 (SNAP-23-(19–211)) reduced the ability of SNAP-23 to bind to GST-VAMP 2 by 60% (Fig. 1B). Deletion of an additional 14 residues from the amino terminus (SNAP-23-(33–211)) eliminated binding of SNAP-23 to VAMP 2, confirming the presence of a VAMP 2 binding region at the extreme amino terminus of SNAP-23. We also examined carboxyl-terminal truncation mutants of SNAP-23 using in vitro binding assays. A deletion mutant lacking the last 23 amino acids of SNAP-23 (SNAP-23-(1–188)) bound very poorly to GST-VAMP 2 (12% binding as compared with wild-type SNAP-23), and further truncations that removed the entire second coiled-coil domain (SNAP-23-(1–140)) completely eliminated the binding of SNAP-23 to VAMP 2.

After observing that the integrity of the extreme amino-
SNAP-23 in Mast Cell Exocytosis

Fig. 1. Mapping of VAMP 2 and syntaxin 4 binding interacting domains of SNAP-23 in vitro. A, schematic of exon/intron organization of SNAP-23 gene. The amino acid boundaries between the exons encoding human SNAP-23 are indicated. The exon structure and amino acid residues listed are based on a complete structure analysis of the gene as described (28). The location of the two putative coiled-coil domains of SNAP-23 are also indicated. B–D, full-length wild-type human SNAP-23-(1–211) or human SNAP-23 deletion mutants (numbers specify amino acids) were radiolabeled by in vitro translation and incubated with 10 μg of GST-VAMP 2 (B) or GST-syntaxin 4 (C) immobilized on glutathione-Sepharose beads for 2 h at 4 °C. After washing, the bound material were analyzed by SDS-PAGE and fluorography. D, equal amounts of radioactive SNAP-25 or SNAP-23 were added to each condition as shown by analyzing an aliquot of the sample input for each condition.

carboxyl-terminal regions of SNAP-23 contributed to its binding to VAMP 2, we examined the importance of these regions in the interaction of SNAP-23 with syntaxin 4. As was seen in the binding assays with VAMP 2, deletion of 18 amino acids from the amino terminus of SNAP-23 almost completely eliminated binding to GST-syntaxin 4 (Fig. 1C). However, unlike the results obtained with VAMP 2, deletion of 23 amino acids from the carboxyl terminus of SNAP-23 did not inhibit its ability to bind syntaxin 4. Removal of the entire second coiled-coil domain of SNAP-23 (SNAP-23-(1–140)) completely prevented the binding of SNAP-23 to syntaxin. Control experiments confirmed that each reaction condition contained similar amounts of SNAP-23 protein (Fig. 1D). These data reveal that the first 18 amino acids of SNAP-23 harbor a major binding site for both syntaxin and VAMP, that the presence of the first coiled-coil alone is insufficient to allow SNAP-23 binding to syntaxin and VAMP, and that the extreme carboxyl terminus of SNAP-23 is not required for the binding of SNAP-23 to syntaxin 4 but is essential for the binding of SNAP-23 to VAMP 2.

Identification of SNAP-23/SNARE Interacting Domains in Vivo—To confirm and extend the results obtained from our in vitro binding studies, we examined the binding characteristics of wild-type SNAP-23 or SNAP-23-(1–188) to syntaxin and VAMP in transfected HeLa cells. In agreement with results obtained in our in vitro binding assays, the association of VAMP 2 with SNAP-23-(1–188) was significantly reduced as compared with the association of VAMP 2 with wild-type SNAP-23 (Fig. 2A). Immunoblotting of the cell extracts confi-

Fig. 2. Binding of wild-type SNAP-23-(1–211) and SNAP-23-(1–188) to SNAREs in vitro. HeLa cells were transiently transfected with empty vector (mock), with wild-type human SNAP-23-(1–211) or human SNAP-23-(1–188) together with FLAG-VAMP 2 (A) or with syntaxin 4 (B). Cell lysates were prepared, and anti-SNAP-23 immunoprecipitates were probed by immunoblotting with anti-VAMP 2 and anti-syntaxin 4 antibodies. Aliquots of the lysates were also analyzed by immunoblotting with specific antibodies recognizing SNAP-23, VAMP 2, and syntaxin 4.
Human SNAP-23 binds syntaxin and VAMP more efficiently than Does Rat SNAP-23—We were intrigued by our data showing that expression of small amounts of human SNAP-23 in mast cells that already express significant amounts of SNAP-23 was able to significantly augment exocytosis, and we set out to identify a molecular mechanism for this effect. Because SNARE complex assembly is essential for exocytosis, we compared the ability of rat SNAP-23 and human SNAP-23 to bind to syntaxin 4 and VAMP 2 using in vitro binding assays. Rat and mouse SNAP-23 (which are 98% identical) bound syntaxin 4 poorly and their binding to VAMP 2 was almost undetectable (Fig. 5). The binding of human SNAP-23 to these SNAREs was considerably better, and the binding of SNAP-25 to each of these GST-SNARE fusion proteins was very efficient. It is important to note that in these binding assays nearly identical amounts of in vitro translated SNAP-23 or SNAP-25 were used, allowing a direct comparison between the binding of these different SNAP-23 family members to GST-SNARE fusion proteins. Data from multiple different in vitro binding assays were compared and show quantitatively that human SNAP-23 binds to syntaxin 4 and VAMP 2 much better than does rat SNAP-23 (Fig. 6). This is in good agreement with a yeast two-hybrid analysis showing that human SNAP-23 bound to syntaxin 4 ~10 times better than did mouse SNAP-23 and that SNAP-25 bound to many different syntaxins very well whereas SNAP-23 did not (11). Thus, the increase in exocytosis from rat mast cells can be explained in part by the expression of a better SNARE binding form of SNAP-23 in these cells.
since even GST-SNAP-23(19–211) fusion proteins did not bind to in vitro translated SNAREs and SNAP-23(19–211) expressed in HeLa cells did not bind to SNAREs in vivo. Furthermore, even full-length SNAP-25 does not have appreciable secondary structure until it binds to syntaxin (7), suggesting that syntaxin binding actually assists in the folding of a relatively unfolded SNAP-25 molecule.

In addition to identifying a SNAP-binding motif in the extreme amino terminus of SNAP-23, a carboxyl-terminal truncation mutant that possessed only the first coiled-coil domain of SNAP-23 did not bind to either syntaxin or VAMP, demonstrating that although the extreme amino terminus of SNAP-23 is necessary for SNAP binding it is not sufficient. The amino terminus of SNAP-25 is known to possess a syntaxin binding site; however, carboxyl-terminal deletion mutants of SNAP-25 that contain only the amino-terminal coiled domain of the protein (e.g. SNAP-25(2–141) and SNAP-25(1–100)) still retain syntaxin binding activity (5, 6). This could reflect the fact that there are 12 heptad repeats in the amino-terminal coiled-coil domain of SNAP-25 and only six in the corresponding region of SNAP-23, thereby limiting the affinity of the SNAP-23 amino-terminal coiled-coil for syntaxin.

We have also identified the carboxyl-terminal 23 amino acids of SNAP-23 as important determinants for binding to VAMP but not to syntaxin. This is in very good agreement with previous studies examining the behavior of SNAP-25 deletion mutants (5–7) and the effects of Botulinum neurotoxin cleavage of SNAP-25 on its SNARE-binding properties (6). To test the functional importance of the SNAP-23 carboxyl-terminal VAMP-binding domain in vivo, we generated rat mast cell lines overexpressing wild-type human SNAP-23 and the human SNAP-23(1–188) carboxyl-terminal deletion mutant. Despite the fact that the total amount of SNAP-23 was minimally altered in these transfectants, expression of human SNAP-23 significantly enhanced exocytosis from these cells. Furthermore, the observed increase in exocytosis required the presence of the carboxyl-terminal 23 amino acids of SNAP-23 as the human SNAP-23(1–188) mutant was unable to support enhanced exocytosis in rat mast cells. This finding is in very good agreement with data showing that treatment of permeabilized Madin-Darby canine kidney cells with Botulinum neurotoxin E, which cleaves ~20 amino acids from the carboxyl terminus of canine SNAP-23, dramatically inhibits transferrin receptor recycling (19) and apical and basolateral membrane traffic (17). These data highlight the importance of the carboxyl-terminal domain of SNAP-23 (encoded by the last exon) in the binding to VAMP 2 and regulating exocytosis.

Despite the similarity in structure and proposed similarity in function for SNAP-23 and SNAP-25, we and others (11) found that SNAP-23 bound to various syntaxins and VAMP 2 much more poorly than did SNAP-25 in vitro. This result is unlikely to be due to our assay system as similar results were obtained in experiments examining the binding of in vitro translated SNAP-23 or SNAP-25 to GST-syntaxin and GST-VAMP fusion proteins and in experiments examining the binding of in vitro translated syntaxins to GST-SNAP-23 and GST-SNAP-25 fusion proteins. In addition, these observations are in excellent agreement with our earlier in vivo experiments demonstrating that SNAP-25 was able to bind to syntaxin more effectively than was SNAP-23 in transfected HeLa cells (30). On the other hand, we also found that human SNAP-23 bound to syntaxin and VAMP 2 better than did rat or mouse SNAP-23, demonstrating that among the SNAP-23 family of proteins there are

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2 V. V. Vaidyanathan, N. Puri, and P. A. Roche, unpublished observations.
3 K. Vogel and P. A. Roche, unpublished observations.
dramatic differences in SNARE binding ability. It is currently unknown if these differences alone can account for enhanced exocytosis in human SNAP-23-expressing rat mast cells or whether additional features of human SNAP-23 can augment exocytosis in rat cells.

Pancreatic β-cells possess SNAP-25, and it has been shown that SNAP-25 is involved in insulin secretion from these cells (31). Interestingly, under conditions in which SNAP-25 is cleaved by Botulinum neurotoxin E and exocytosis is diminished, human SNAP-23 (which is not cleaved by this toxin) can rescue exocytosis only if it is expressed at extraordinarily high levels (22). In light of our current work, we interpret this result by proposing that replacement of SNAP-25 (an excellent SNARE-binder) by human SNAP-23 (a moderate SNARE-binder) would require very large amounts of human SNAP-23. On the other hand, enhancing exocytosis from mast cells that use rat SNAP-23 (a poor SNARE-binder) by introducing human SNAP-23 (a moderate SNARE-binder) would require only small amounts of human SNAP-23. Our data presented here, together with the functional results of Sadoul et al. (22) are in very good agreement with this hypothesis. Although we had hoped to overexpress the excellent SNARE-binder SNAP-25 in rat mast cells, we were unable to isolate healthy RBL transfectants expressing significant amounts of SNAP-25. This could be due to toxicity of overexpressed SNAP-25 in these cells, and in agreement with this we were also unable to isolate stable RBL transfectants expressing very large amounts of human SNAP-23. We conclude from these studies that the efficiency of exocytosis is regulated in part by the ability of SNARE complexes to form in living cells since compensation for a loss of SNAP-25 requires expression of large amounts of human SNAP-23 and expression of even small amounts of human SNAP-23 can potentiate secretory processes in cells possessing rat SNAP-23.

The carboxyl terminus of SNAP-23 has previously been implicated in GLUT4 trafficking in 3T3-L1 adipocytes. Microinjection of a carboxyl-terminal peptide of SNAP-23 into 3T3-L1 cells inhibited GLUT4 trafficking (14), although the mechanism by which this peptide inhibited translocation was not explored. In a related study, infection of 3T3-L1 cells with a recombinant virus encoding mouse SNAP-23 (1–202) also inhibited GLUT4 trafficking (15), yet in this study even wild-type SNAP-23 inhibited GLUT4 trafficking. Our study is the first to reveal that the introduction of human SNAP-23 into regulated secretory cells actually augments exocytosis, that this form of (human) SNAP-23 binds to SNARE proteins better than endogenous (rat) SNAP-23, and that the increase in exocytosis can be directly attributed to the integrity of the carboxyl-terminal VAMP-binding domain of SNAP-23. These data also highlight the similarities between the mechanism of GLUT4 translocation in fat cells and granule exocytosis in mast cells.

Besides the current work, several studies have shown a direct relationship between SNARE complex assembly and fusion. In the neuroendocrine PC12 cell line Ca2+ was found to enhance SNARE complex assembly and subsequently drive membrane fusion (32). Studies in adrenal chromaffin cells have suggested that trans-SNARE complexes (i.e. complexes of v-SNAREs with t-SNAREs from opposing membranes) exist in two interconvertible states: a “tight” state that mediates the fast component of release and a “loose” state that mediates a sustained slow phase (33). It is interesting to note that, in general, the kinetics of non-neuronal exocytosis are far slower than neuronal exocytosis. Because non-neuronal exocytosis involves SNARE complexes containing SNAP-23 and not SNAP-25, it is possible that at least one contributing factor to the diminished kinetics of exocytosis in non-neuronal cells is the presence of the “weak” SNAP-23 SNAP-23 in secretory trans-SNARE complexes.

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