Expression and localisation of synaptotagmin isoforms in endocrine β-cells: their function in insulin exocytosis

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SUMMARY

Exocytosis of insulin containing Large Dense Core Vesicles (LDCVs) from pancreatic β-cells and derived cell lines is mainly controlled by Ca\textsuperscript{2+}. Several lines of evidence have demonstrated a role of the Ca\textsuperscript{2+}- and phospholipid-binding protein synaptotagmin (syt) in this event. Synaptotagmins form a large protein family with distinct affinities for Ca\textsuperscript{2+} determined by their two C\textsubscript{2} domains (C\textsubscript{2A}/B). Except for the well-characterized isoforms I and II, their role is still unclear. We have used here insulin-secreting cells as a model system for LDCV exocytosis to gain insight into the function of synaptotagmins. Immunocytochemical analysis revealed that of the candidate Ca\textsuperscript{2+} sensors in LDCV exocytosis, syt III was not expressed in primary β-cells, whereas syt IV was only found adjacent to the TGN. However, syt V-VIII isoforms were expressed at different levels in various insulin-secreting cells and in pancreatic islet preparations. In streptolysin-O permeabilized primary β-cells the introduction of recombinant peptides (100 nM) corresponding to the C\textsubscript{2} domains of syt V, VII and VIII, but not of syt III, IV or VI, inhibited Ca\textsuperscript{2+}-evoked insulin exocytosis by 30% without altering GTP\textsubscript{S}-induced release. Our observations demonstrate that syt III and IV are not involved in the exocytosis of LDCVs from primary β-cells whereas V, VII and VIII may mediate Ca\textsuperscript{2+}-regulation of exocytosis.

Key words: Exocytosis, Large dense core vesicle (LDCV), Ca\textsuperscript{2+}, Endocrine cell, Insulin

INTRODUCTION

The release of neurotransmitters and peptide hormones proceeds by exocytosis, that is, fusion between the membranes of the corresponding vesicles and the plasma membrane (Jahn and Sudhof, 1999). The release of potent substances must be tightly controlled and in most systems an increase in cytosolic Ca\textsuperscript{2+} constitutes the major stimulatory signal for exocytosis (Burgoyne and Morgan, 1998). During the last decade a number of proteins have been identified which are required for this process. A heterotrimeric complex of proteins, the so-called SNARE proteins, exerts a major role (Rothman and Sollner, 1997). The complex encompasses synaptobrevin/VAMP, which is located on exocytotic vesicles, as well as SNAP-25 and syntaxin, mainly present on the plasma membrane. Assembly and disassembly of this complex seems to be necessary for exocytosis.

Ca\textsuperscript{2+}-dependent regulation may be imposed through a class of vesicle proteins, the synaptotagmins (syt), which interact with both the SNARE proteins syntaxin and SNAP-25, and with membrane phospholipids (Fernandez-Chacon and Sudhof, 1999). 13 isoforms of synaptotagmin are presently known and are predicted to exhibit several common structural features (Marquiéze et al., 2000). The best-characterised isoform, syt I, contains a short intravesicular N terminus followed by a single transmembrane domain and a region of variable length connecting to two C\textsubscript{2} domains (C\textsubscript{2A}/B). These C\textsubscript{2} domains were first characterised in protein kinase C, where they mediate phospholipid binding at micromolar concentrations of free Ca\textsuperscript{2+}. In addition, binding of Ca\textsuperscript{2+} to the C\textsubscript{2}A domain alters its electrostatic potential and thus permits binding to the SNARE protein syntaxin at Ca\textsuperscript{2+} concentrations above 100 μM (Rizo and Sudhof, 1998). These concentrations of the cation are in accordance with levels of cytosolic Ca\textsuperscript{2+} reported for exocytosis from bipolar retinal neurones (Heidelberger et al., 1994). The implication of synaptotagmin as a Ca\textsuperscript{2+} sensor in exocytosis has been strengthened by the observations that cytosolic applications of recombinant peptides corresponding to the C\textsubscript{2} domains of syt V, VII and VIII, but not of syt III, IV or VI, inhibited Ca\textsuperscript{2+}-evoked insulin exocytosis by 30% without altering GTP\textsubscript{S}-induced release. Our observations demonstrate that syt III and IV are not involved in the exocytosis of LDCVs from primary β-cells whereas V, VII and VIII may mediate Ca\textsuperscript{2+}-regulation of exocytosis.
whereas others such as syt VI-IX and XI are seemingly more widely distributed (Marquez et al., 2000). Moreover, only the isoforms I-V and VII bind phospholipids at low micromolar Ca2+. The interaction with syntaxin is apparent either at low (III and VII) or high (I, II and V) micromolar levels of Ca2+ or is absent (Li et al., 1995). Their differential distribution and biochemical characteristics have contributed to the notion that synaptotagmins may function in a variety of distinct membrane fusion events (Marquez et al., 2000), although their precise role remains a matter of debate. Indeed, conflicting results on the localisation and function of the isoforms III (Butz et al., 1999; Brown et al., 2000) and IV (Ferguson et al., 1999; Berton et al., 2000; Ibata et al., 2000) have been reported.

To study the putative role of synaptotagmin isoforms in exocytosis of peptide-containing large dense core vesicles (LDCVs) we have used pancreatic β-cells and derived cell lines (Lang, 1999). These preparations represent a valid model as the use of permeabilised cells permits the study of exocytosis (Lang et al., 1997b). Under these conditions the release of insulin is stimulated upon an increase in intracellular Ca2+ or the addition of the slowly hydrolyzable GTP analogue GTPγS (Vallar et al., 1987). Ca2+-stimulated exocytosis of insulin requires functional SNARE proteins as shown by the use of clostridial neurotoxins (Lang et al., 1997c; Martin et al., 1995; Regazzi et al., 1996; Sadoul et al., 1995). We have previously demonstrated a role for the two synaptotagmin isoforms syt I and II, in two β-cell derived cell lines in exocytosis evoked by Ca2+, but not by GTPγS (Lang et al., 1997a). In view of the different isoforms known we have now set out to characterise the localisation and involvement of syt III and IV in this model system of LDCV exocytosis as well as the putative role of syt V-VIII.

MATERIALS AND METHODS

Materials
Streptolysin-O was produced and purified as described (Weller et al., 1996). The generation and testing of antibodies against the C2A domains of syt III or syt IV have been described previously (Fukuda et al., 1999; Ibata et al., 2000). Plasmids encoding GST-tagged synaptotagmin I-VIII were generously donated by Dr T. E. Südhof (Dallas, USA). GST-tagged recombinant proteins purified according to the instructions of the manufacturer and in the absence of detergents were >95% pure, as judged by Coomassie Blue stained SDS-PAGE gels.

Cell culture and release studies
Culture of the insulin-secreting cell lines HIT-T15 or MIN6 and preparation of primary islet cells were from male Wistar rats as described previously (Lang et al., 1998). Preparation of intracellular buffers and permeabilisation with streptolysin-O were as described (Lang et al., 1997a,b). 100 nM GST-syt-C2A recombinant peptides were added to the permeabilised cells in intracellular buffer at 0.1 μM free Ca2+. After 10 minutes preincubation, this solution was washed for 1 hour and 5% BSA. Primary antibodies were then washed three times for 5 minutes with PBS-1% BSA, and incubated with secondary antibodies in PBS containing 0.1% saponin (Sigma, Buchs, Switzerland) and 5% BSA. Primary antibodies were added for 1-4 hours at room temperature or overnight at 4°C. Cells were then washed three times for 5 minutes with PBS-1% BSA, and incubated with secondary antibodies in PBS containing 0.1% saponin and 5% BSA for 1 hour. After three washes with PBS for 5 minutes, cells were mounted on glass slides using a mounting medium (Vector Lab., Burlingame, USA). Confocal laser microscopy was performed with a Zeiss LSM 410 inverse laser scan microscope equipped with an argon and helium-neon laser (Zürich, Switzerland). Primary antibodies were diluted as indicated in the figure legends.

Statistical analysis
Data are presented as mean ± s.e.m. from experiments performed independently on at least three different cell preparations. Statistical analysis was performed by Student’s two-tailed t-test for unpaired data (2P).

RESULTS
The synaptotagmin protein family contains a number of isoforms and some of them have been implicated in the regulation of insulin exocytosis (Brown et al., 2000; Lang et al., 1997a; Mizuta et al., 1994; Mizuta et al., 1997). Syt III has previously been reported to play a role as a Ca2+ sensor in the exocytosis of secretory granules (Brown et al., 2000; Mizuta et al., 1997), whereas syt IV was found to be important in the exocytosis of LDCVs and synaptic vesicles (Ferguson et al., 1999; Littleton et al., 1999; Thomas et al., 1999). Therefore we studied their subcellular distribution to determine whether their localisation permits them to intervene in exocytosis.

First we examined the specificity of the antibodies directed against syt III or IV as used here. Whereas the anti-synaptotagmin III antibody was highly specific for the syt III-C2A peptide (Fig. 1A), the anti-synaptotagmin IV antibody demonstrated some minor crossreaction with other recombinant proteins, mainly syt II. We further determined the specificity by testing whether the binding could be blocked by the corresponding recombinant peptide. As shown in Fig. 1B, the syt III peptide, but not the syt IV peptide, inhibited the binding of the anti-syt III antibody on western blots from rat brain homogenates. Similarly, the reactivity of the anti-syt IV
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antibody was blocked by the syt IV peptide, but not by the syt III. Using these antibodies in western blots from cell homogenates (Fig. 1C) we found immunoreactive bands of the corresponding molecular mass for syt III mainly in MIN6 cells and to a lesser extent in RINm5F and HIT-T15 cells, whereas no staining was detectable in INS-1 or primary islet cells. A 45 kDa band corresponding to syt IV was found in all cell lines tested except for HIT-T15 cells.

To further establish the distribution of these two synaptotagmin isoforms we resorted to confocal laser immunofluorescence (Fig. 2). Insulin-secreting β-cells represent the majority of primary islet cells; however, such a preparation also contains somatostatin-secreting δ-cells and glucagon-secreting α-cells. It is therefore important to identify precisely the cell type involved. Within the primary islet cells the anti-synaptotagmin III antibody stained a few cells which were not, however, β-cells as they were not recognized by an antibody against insulin (Fig. 2a). In contrast, these cells were stained with an antibody against somatostatin, suggesting that syt III is only expressed in pancreatic δ-cells (data not shown). The presence of syt III on secretory vesicles has been reported for the insulin-secreting cell line MIN6 (Mizuta et al., 1997). Our antibody did indeed detect immunoreactive material with a granular distribution in this cell line (Fig. 2c). However, close inspection revealed only minor colocalisation between the immunoreactivity for syt III and for insulin (Fig. 2d). Taking into account the resolution obtained by confocal microscopy, these data suggest that syt III is most unlikely to be expressed on insulin-containing granules.

To obtain information on the targeting of syt III we performed transient expression in both primary islet cells and in HIT-T15 cells, as in the latter we could not reliably detect syt III by immunofluorescence. Primary β-cells transiently expressed syt III upon transfection. However, the immunoreactivity was mainly present at the level of the plasma membrane (Fig. 2b, green) and did not coincide with insulin (Fig. 2b, red). To identify transiently transfected HIT-T15 cells, they were cotransfected with a plasmid encoding eGFP. As shown in Fig. 2e, synaptotagmin III was again solely found at the plasma membrane and not on intracellular structures.

In the case of syt IV, a strong immunostaining was found in a few cells in preparations of primary islet cells (Fig. 3a, green). This strong signal was confined to glucagon-secreting α-cells (Fig. 3c) and associated with structures distinct from secretory granules. A fainter signal was observed in all cells (Fig. 3b). Costaining with an antibody against TGN38, a marker for the trans-Golgi network, revealed that syt IV was mainly found within or adjacent to the TGN (Fig. 3b). A similar perinuclear distribution was apparent in MIN6 cells and again no colocalisation with secretory granules was apparent. In HIT-T15 cells only a very faint signal was observed at a location compatible with the TGN (data not shown).

As syt III and syt IV are not expressed on secretory vesicles we also investigated the relative distribution of the synaptotagmin isoforms V-VIII in these various cell lines. To this end northern blots were hybridised with specific probes and compared to the expression of actin. As shown in Fig. 4, all isoforms assayed are expressed in insulin-secreting cells, although at different levels. The results suggest that synaptotagmins V-VIII are widely expressed in these cells and may eventually be involved in the exocytosis of secretory granules.

We employed next a functional approach to gain insight into the potential role of synaptotagmin isoforms. The C2A/B domains are known to be important in the function of synaptotagmins and their binding to other components of the exocytotic machinery (Fernandez-Chacon and Sudhof, 1999; Rizo and Sudhof, 1998). Consequently, it has been shown that
the cytosolic application of the corresponding domains of syt I blocks the exocytosis of LDCVs in PC12 cells (Elferink et al., 1993). We therefore examined the effect of these domains of recombinant syt I-VIII on insulin exocytosis in streptolysin-O permeabilized primary islet cells (Fig. 5). We also compared the stimulation of insulin exocytosis by Ca$^{2+}$ with the stimulation by the slowly hydrolysable GTP analogue GTP$\gamma$S, as the guanine nucleotide exerts its effect independent of Ca$^{2+}$ (Vallar et al., 1987). In primary islet cells, only syt V, VII and VIII were effective and as expected, the GTP$\gamma$S-induced effects

**Fig. 2.** Subcellular localization of endogenous and transiently expressed synaptotagmin III in primary islet, MIN6 and HIT-T15 cells. Islet cells: cells grown on coverslips were fixed, permeabilised and incubated with the anti-syt III polyclonal antibody (1:200) and monoclonal anti-insulin antibody (1:200). Anti-syt III binding was revealed with an FITC-coupled second antibody (green), anti-insulin binding with a TRITC-coupled second antibody (red). (a) Native cells; (b) cells transiently expressing synaptotagmin III. MIN6 cells: primary antibodies bound to native syt III or insulin were visualized with fluorophore-coupled secondary antibodies; (c) TRITC, anti-syt III; (d) FITC anti-syt III, TRITC, anti-insulin). HIT-T15: cells were transiently cotransfected with plasmids expressing syt III and plasmids expressing eGFP. Primary antibodies bound to native syt III were visualized with TRITC-coupled secondary antibodies. Immunofluorescence and phase-contrast images are given in the upper and lower panels, respectively. Bars, 5 μm.

**Fig. 3.** Subcellular localization of synaptotagmin IV in pancreatic islets, MIN6 and HIT-T15 cells. Islet cells: cells were permeabilised and fixed. Staining for syt IV (a-c) was revealed with an FITC-coupled second antibody (green), costaining for insulin (a), anti-TGN (b) or anti-glucagon (c) was revealed with a TRITC-coupled second antibody (red). MIN6: staining for syt IV (d) was revealed with a FITC-coupled second antibody (green), costaining for insulin was revealed with a TRITC coupled second antibody (red).
Synaptotagmin isoforms in insulin exocytosis were not altered. We also performed experiments in the hamster insulinoma cell line HIT-T15: the peptides corresponding to syt I or II inhibited Ca\textsuperscript{2+}-induced insulin release by 35.7±4.1% and 28±6.2%, respectively (2P<0.05) without altering GTP\gammaS-evoked release (data not shown). These observations are in line with our previous results in HIT-T15 cells obtained by the use of site-specific antibodies or point mutations (Lang et al., 1997a). In addition, the recombinant C2 A/B domains of syt VI, VII and VIII specifically inhibited Ca\textsuperscript{2+} induced exocytosis (28.4±4.7%, 29.8±5.9% and 23.4±4.3%, respectively; 2P<0.05) but did not significantly alter GTP\gammaS mediated release. This suggests that syt VII and VIII may have a general function in insulin exocytosis, whereas the effect of the isoforms I, II, V and VI are cell-type dependent.

DISCUSSION

Within the family of the Ca\textsuperscript{2+}-binding protein synaptotagmin, the two isoforms syt I and syt II play a role in the exocytosis of LDCVs and SVs (Elferink et al., 1993; Geppert et al., 1994; Littleton et al., 1994; Lang et al., 1997a). We have previously shown that syt I and II are operational in endocrine exocytosis probably involving their capacity to bind phospholipids. However, in contrast to derived cell lines, primary β-cells do not express syt I or II, raising the question about the nature of the relevant isoform present in primary cells (Lang et al., 1997a). Moreover, a considerable number of additional isoforms has been identified during recent years and their respective roles still remain unclear (reviewed in Marqueze et al., 2000). Our study has therefore addressed the expression of synaptotagmin isoforms in a model system of LDCV exocytosis, namely pancreatic β-cells or derived cell lines, to investigate their potential implication in Ca\textsuperscript{2+} evoked exocytosis.

Syt III has been proposed to play an essential role in regulated exocytosis from endocrine β-cells (Brown et al., 2000; Mizuta et al., 1994; Mizuta et al., 1997), whereas syt IV regulates exocytosis in Drosophila neurons and neuroendocrine PC12 cells (Ferguson et al., 1999; Littleton et al., 1999; Thomas et al., 1999). In our system we could not find any evidence for their expression on relevant organelles or a role in the exocytosis of peptide hormone containing LDCVs. Clearly the investigation of isoforms always implies the problem of crossreactivity. Phylogenetic analysis reveals a close relationship between syt III and syt V, VI or X as well as between syt IV and syt XI (Marqueze et al., 2000). The C\textsubscript{2}A domain of syt III used as an antigen exhibits 74% identity to the corresponding domains of syt V, syt VI or syt X and less than 65% identity to other synaptotagmin isoforms. It should be noted that the antibodies used in our study had been preabsorbed to syt VI to remove crossreactivity and the specificity has been tested against syt I-VIII (our results) and syt IX-XI (Fukuda et al., 1999; Ibata et al., 2000). We therefore assume that any crossreactivity with these isoforms is unlikely.

Although high expression levels of syt III mRNA were
evident in the rather differentiated cell line INS-1 and in concordance with a previous study in MIN6 cells (Mizuta et al., 1994); only low levels were found in pancreatic islets. Indeed, the level of expression in islets was comparable to or even lower than RINm5F cells. The latter are poorly granulated and contain 100-fold less insulin than islet cells (Wollheim et al., 1990). A scarcely expressed protein may, of course, fulfill an important function. However, immunohistochemistry demonstrated that in primary islet cells syt III is only expressed in somatostatin-secreting δ-cells, but not in β-cells. Therefore, the low level of expression in islets is most likely to reflect its expression in somatostatin-secreting cells, which represent less than 10% of islet cells. It is noteworthy that the distribution in δ-cells was granular, but did not match with somatostatin-containing secretory granules (not shown). Similar to a previous report (Mizuta et al., 1997) we found a granular distribution of syt III in MIN6 cells, from which it was originally been cloned. However, these syt III-containing structures overlapped only partially with insulin-containing vesicles. Finally transiently expressed syt III in pancreatic β-cells or HIT-T15 cells was almost exclusively localised at the plasma membrane. In nerve terminals the majority of syt III is also not expressed on synaptic vesicles but on the plasma membrane or, alternatively, on a still undefined compartment (Butz et al., 1999). Although overexpression may eventually result in a saturation of the sorting machinery, such an artifact is not responsible for the observed localisation as staining of the correct compartment should still be observed. However, we could not detect any evidence for localisation outside the plasma membrane or any evidence for intracellular aggregation. Hence, syt III seems in general to be directed to the plasma membrane. The different expression pattern in MIN6 and δ-cells may be explained by the presence of a cell-specific cofactor, which routes syt III to an organelle with a granular distribution.

We were also unable to localise syt IV on any exocytotic compartment, in contrast to reports of pheochromocytoma PC12 cells and Drosophila brain (Ferguson et al., 1999; Littleton et al., 1999; Thomas et al., 1999). In accordance with two other reports on localisation of syt IV in hippocampal neurons and PC12 cells (Berton et al., 2000; Ibata et al., 2000), a structure adjacent to the TGN was immunostained in all cells examined. Interestingly the expression of syt IV has recently been demonstrated on immature granules with subsequent retrieval during maturation (Eaton et al., 2000). In contrast to β-cells, syt IV was strongly expressed in glucagon-secreting primary α-cells, but again on a compartment distinct from peptide hormone-containing LDCVs. Immunopurification and in vitro binding studies demonstrated an interaction between syt I and syt IV, which may be of functional importance (Littleton et al., 1999). In view of the distinct distribution of syt I in derived β-cells, such an interaction may eventually only occur at the level of the TGN, and certainly not at the sites of exocytosis. Finally, we were unable to observe any prominent staining in somatostatin-containing δ-cells, in contrast to a previous report (Brown et al., 2000). As syt IV presents an immediate early gene product (Vician et al., 1995), we cannot exclude the possibility that the discrepancy between its expression in δ- and α-cells may reside in the use of fresh versus cultured islets.

The functional assay used also argues against a role for syt III or IV in insulin exocytosis. The assay is based on an early observation that cytosolic application of C2A/B domains of syt I inhibits Ca2+ stimulated exocytosis from PC12 cells (Elferink et al., 1993). The same approach has also been used recently to investigate the putative function of syt VII in the exocytosis of lysosomes (Martinez et al., 2000). The use of specific peptides or antibodies offers the advantage of an acute intervention, thus avoiding problems inherent in the selection of stable clones. The mechanisms of how these peptides inhibit are not completely clear but may involve two distinct mechanisms. First, synaptotagmins undergo homodimerisation, which may be required for their function (Fukuda et al., 1999; Fukuda and Mikoshiba, 2000), and heterodimerisation, which may even occur at low micromolar concentrations of Ca2+ (Osborne et al., 1999). Binding of endogenous synaptotagmins to recombinant C 2 A/B domains of syt I-VIII (syt I-VIII; GST, GST M or 100 μM GTPγS (at 0.1 μM Ca2+) in the presence of GST fusion peptides corresponding to the C 2 A/C 2 B domains of synaptotagmin isoforms I-VIII (syt I-VIII; GST, GST alone). Insulin secretion was measured by radioimmunoassay and results are expressed as a percentage of inhibition of net release as compared to the absence of peptides. *2P<0.05 as compared to GST alone.

Fig. 5. Differential effects of synaptotagmin C2A/B peptides on insulin release from streptolysin-O permeabilized primary rat islet cells. Primary islet cells were permeabilized with streptolysin-O and subsequently exposed to basal or stimulatory concentrations of Ca2+ (0.1 μM or 10 μM) or 100 μM GTPγS (at 0.1 μM Ca2+) in the presence of GST fusion peptides corresponding to the C2A/C2B domains of synaptotagmin isoforms I-VIII (syt I-VIII; GST, GST alone). Insulin secretion was measured by radioimmunoassay and results are expressed as a percentage of inhibition of net release as compared to the absence of peptides. *2P<0.05 as compared to GST alone.

The specificity of the approach used here is underlined by several findings. First, the effect of recombinant peptides was...
restricted to Ca\textsuperscript{2+}-induced exocytosis, whereas GTP\textsuperscript{S} evoked release remained unchanged. Indeed, GTP\textsuperscript{S}-evoked release is known to proceed independently of Ca\textsuperscript{2+} (Vallar et al., 1987) or synaptotagmin, as indicated by the use of site-specific antibodies and mutants (Lang et al., 1997a). Second, in concordance with previous experiments using site-specific antibodies and mutants (Lang et al., 1997a), the peptides corresponding to syt I and II inhibited exocytosis in HIT-T15 cells. In contrast, these peptides did not alter exocytosis in primary β-cells that do not express these proteins.

Syt I and II are known to interact in vitro with the SNARE protein syntaxin (Li et al., 1995). This interaction exhibits an EC\textsubscript{50} for Ca\textsuperscript{2+} between 200 and 500 μM, in accordance with subplasmalemmal Ca\textsuperscript{2+} concentrations occurring in neuroexocytosis (Heidelberger et al., 1994). Both HIT-T15 cells and primary β-cells express syntaxin I and require its function for exocytosis (Lang et al., 1997c; Martin et al., 1995). The failure of the recombinant C\textsubscript{2A}/B domains of syt I and II to inhibit exocytosis from primary β-cells suggests that Ca\textsuperscript{2+}-dependent interactions with syntaxin may not be the major mechanism probed for with the recombinant peptides. Although the true Ca\textsuperscript{2+} dependency of the interaction between syntaxin and synaptotagmin in-vivo is unknown, exocytosis from permeabilised cells was tested under conditions providing the maximal stimulation in our system as measured by biochemical means, that is 10 μM Ca\textsuperscript{2+}. At these levels of Ca\textsuperscript{2+}, syt III, V and VII bind to phospholipids (Li et al., 1995). Disturbance of this interaction of synaptotagmin dimerisation or of binding to still unknown factors may form the basis for the effect of recombinant C\textsubscript{2A}/B domains corresponding to specific isoforms.

Our functional data argue strongly against the possibility that syt III or IV play a role in Ca\textsuperscript{2+} stimulated exocytosis, at least in primary β-cells and clonal HIT-T15 cells. In contrast, we observed significant effects in the case of syt V, VIII and VII in primary β-cells, whereas syt VI-VIII were active in HIT-T15 cells. The differential activities of the recombinant peptides syt V and VI again underscore the cell-type specificity, as also observed for syt I and II. Syt V-VIII are expressed in primary islet cells and clonal cell lines; we still do not know whether they are present in β-cells and on which organelle they reside. Syt V and VII both exhibit Ca\textsuperscript{2+} sensitivity in their binding to phospholipids and syntaxin I (Li et al., 1995). Whereas the subcellular localisation and precise organelle they reside. Syt V and VII both exhibit Ca\textsuperscript{2+} sensitive, may constitute promising candidates for further investigation.

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