Synaptotagmin III/VII Isoforms Mediate Ca\(^{2+}\)-induced Insulin Secretion in Pancreatic Islet \(\beta\)-Cells

Zhiong Gao, John Reavey-Cantwell, Robert A. Young, Patricia Jegier, and Bryan A. Wolf

From the Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Received for publication, May 18, 2000, and in revised form, July 24, 2000

Published, JBC Papers in Press, August 9, 2000, DOI 10.1074/jbc.M004284200

Synaptotagmins (Syt) play important roles in Ca\(^{2+}\)-induced neuroexocytosis. Insulin secretion of the pancreatic \(\beta\)-cell is dependent on an increase in intracellular Ca\(^{2+}\); however, Syt involvement in insulin exocytosis is poorly understood. Reverse transcriptase-polymerase chain reaction studies showed the presence of Syt isoforms III, IV, V, and VII in rat pancreatic islets, whereas Syt isoforms I, II, III, IV, V, VII, and VIII were present in insulin-secreting \(\beta\)TC3 cell. Syt III and VII proteins were identified in rat islets and \(\beta\)TC3 and RINm5F \(\beta\)-cells by immunoblotting. Confocal microscopy showed that Syt III and VII co-localized with insulin-containing secretory granules. Two-fold overexpression of Syt III in RINm5F \(\beta\)-cell (Syt III cell) was achieved by stable transfection, which conferred greater Ca\(^{2+}\) sensitivity for exocytosis, and resulted in increased insulin secretion. Glyceroldehyde + carbachol-induced insulin secretion in Syt III cells was 2.5-fold higher than control empty vector cells, whereas potassium-induced secretion was 6-fold higher. In permeabilized Syt III cells, Ca\(^{2+}\)-induced and mastoparan-induced insulin secretion was also increased. In Syt VII-overexpressing RINm5F \(\beta\)-cells, there was amplification of carbachol-induced insulin secretion in intact cells and of Ca\(^{2+}\)-induced and mastoparan-induced insulin secretion in permeabilized cells. In conclusion, Syt III/VII are located in insulin-containing secretory granules, and we suggest that Syt III/VII may be the Ca\(^{2+}\) sensor or one of the Ca\(^{2+}\) sensors for insulin exocytosis of the \(\beta\)-cell.

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Insulin exocytosis from the \(\beta\)-cell of the islets of Langerhans is stimulated by various physiological secretagogues that include glucose, amino acids, and receptor-mediated agonists such as acetylcholine, cholecystokinin, and glucagon like-peptide 1 (1–7). A common mechanism of action for these secretagogues is to cause an increase in cytosolic Ca\(^{2+}\). Elevation of intracellular Ca\(^{2+}\) is due to an influx of extracellular Ca\(^{2+}\) through voltage-dependent L-type Ca\(^{2+}\) channel and/or mobilization of intracellular Ca\(^{2+}\) from the endoplasmic reticulum (8–17). However, the mechanisms by which Ca\(^{2+}\) induces insulin granule fusion with the plasma membrane of \(\beta\)-cell remain unclear (1, 16, 18, 20, 21).

Synaptotagmin (Syt) is a family of membrane proteins initially found to be expressed in brain. At the present, 11 members of Syt have been identified (22, 23). The Syt molecule has a single transmembrane domain and two Ca\(^{2+}\) regulatory C\(_2\) domains. The C\(_2\) domains mediate Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent interactions with target molecules that may regulate membrane fusion and membrane budding reactions (24, 25). Literature concerning the expression and functions of Syt in pancreatic \(\beta\)-cell is very limited and contradictory. In an earlier study (26), Syt was found in the non-\(\beta\)-cell of the islet mantle, but not in the \(\beta\)-cell, using a non-isoform-specific antibody, and the mRNAs of Syt A and B were absent in mouse pancreatic \(\beta\)-cell and RINm5F cells as demonstrated by in situ hybridization. Recently, the mRNA and protein of Syt isoforms I and II (27) were found in insulin-secreting \(\beta\)-cell lines RINm5F, INS-1, and HIT-T15. It was reported that Syt I, II (27), and Syt III proteins (28) are localized mainly in insulin-containing secretory granules. However, only the mRNA but not the protein was detected in primary islet \(\beta\)-cells (27). In other studies, Syt III mRNA was present in MIN6, RINm5F, HIT-T15, and \(\beta\)TC6-f7 (29) cells and pancreatic islets (28), and the protein expression of Syt III in MIN6 cell and pancreatic islets was confirmed by one group (28) but not by another (29).

The aims of the current study were to examine the expression of various Syt isoforms in pancreatic islets as well as insulin-secreting \(\beta\)-cell lines, the subcellular localization of Syt, and the functional role of Syt in insulin exocytosis using a \(\beta\)-cell line overexpressing Syt.

EXPERIMENTAL PROCEDURES

Insulin-secreting \(\beta\)-Cell Lines and Islet Preparation—The insulin-secreting mouse \(\beta\)-cell line \(\beta\)TC3 and rat \(\beta\)-cell line RINm5F were maintained in culture as described previously (17, 30–33). In brief, cells were maintained in RPMI 1640 containing 11 mM glucose (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 100 units/ml penicillin, 50 \(\mu\)g/ml streptomycin and incubated at 37 °C in a 5% \(\text{CO}_2\), 95% air humidified incubator. Isolated islets of Langerhans were prepared from Harlan Sprague-Dawley rats by collagenase digestion and Ficoll purification as described previously (34–36). In some experiments, single islet cells were prepared by digesting islets in Ca\(^{2+}\)-free and Mg\(^{2+}\)-free PBS containing 1 mM EGTA, 1% BSA, and trypsin at 30 °C for 10 min with continuous trituration (37).

Generation of Stable \(\beta\)-Cells Overexpressing Synaptotagmin III or VII—The plasmids with the insert of Syt III or Syt VII cDNA under the control of cytomegalovirus promoter were generously provided by

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* This work was supported by National Institutes of Health Grants DK43354 and DK49814. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Both authors should be considered first authors.

§ To whom correspondence should be addressed: University of Pennsylvania School of Medicine, Dept. of Pathology & Laboratory Medicine, 230 John Morgan Bldg., 3620 Hamilton Walk, Philadelphia, PA 19104-6082. Tel.: 215-898-0025; Fax: 215-573-2266; E-mail: wolfe@mail.med.upenn.edu.

This paper is available on line at http://www.jbc.org

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RINm5F cells were washed twice with ice-cold PBS containing 1% BSA. Coverslips and cultured for 1–2 days. Coverslips with islet cells or once with 1 ml of RPMI 1640 medium, and then plated on coverslips and antibodies described above (17, 43).

Preparation of Synaptotagmin C-terminal Peptides and Antibodies—The first strand cDNA from 2 μg of total RNA was synthesized using SuperscriptTM II Reverse Transcriptase (purchased from Life Technologies, Inc.) and oligo(dT) 15 primer (500 μg/ml, Life Technologies, Inc.). RNA was denatured at 70 °C for 10 min, followed by a reverse transcription at 42 °C for 60 min and then 75 °C for 10 min.

The primers were designed and synthesized based on the published gene sequence as shown in Table I. The PCR was carried out in a thermal cycler (model 480, PerkinElmer Life Sciences) with an initial denaturation step at 94 °C for 3 min, subjected to 35 cycles of denaturation (94 °C for 1 min), annealing (55 °C for 2 min), elongation (72 °C for 3 min), and a final elongation step at 72 °C for 7 min. PCR products were visualized by 1% agarose gel electrophoresis with ethidium bromide staining.

RT-PCR Detection of mRNA—Total RNA was extracted from rat pancreatic islets and bTC3 and RINm5F cells, using the micro-scale total RNA separator kit (CLONTECH) following the manufacturer’s instructions, as described previously (41, 42). The purity and the yield of isolated RNA were determined by monitoring absorbance at 260 and 280 nm. The integrity of the RNA was confirmed by performing agarose gel electrophoresis. All RNA samples were treated with DNase to eliminate genomic DNA contamination before RT-PCR. The primers were designed and synthesized based on the published mRNA sequence as shown in Table I. The PCR was carried out in a thermal cycler (model 480, PerkinElmer Life Sciences) with an initial denaturation step at 94 °C for 3 min, subjected to 35 cycles of denaturation (94 °C for 1 min), annealing (55 °C for 2 min), elongation (72 °C for 3 min), and a final elongation step at 72 °C for 7 min. PCR products were visualized by 1% agarose gel electrophoresis with ethidium bromide staining.

**TABLE I**

| Isoform | F or R* | Starting site | Product size | Primer sequence |
|---------|---------|--------------|--------------|-----------------|
| I       | F       | 600          | 640          | 5’-ACTGAGCCAGCCAGTCTCGG-3’ |
| II      | R       | 1240         | 784          | 5’-ATGTGCTGGTCAGGACGCG-3’ |
| III     | F       | 1360         | 691          | 5’-AGAACAGATGACGACGCCG-3’ |
| IV      | F       | 109          | 613          | 5’-TCTGTCATCCTCAGTGAGG-3’ |
| V       | R       | 808          | 613          | 5’-GAGCTAGCCCTGGGTTCTC-3’ |
| VI      | F       | 1080         | 752          | 5’-GAGAAGCAGATGACGCCG-3’ |
| VII     | R       | 161          | 711          | 5’-AGAAGCAGATGAGAACCAG-3’ |
| VIII    | F       | 913          | 619          | 5’-TCACTGGCTCTGGTTCTG-3’ |
| I, set 2| F       | 1771         | 711          | 5’-TCACTGGCTCTGGTTCTG-3’ |
| III, set 2| F   | 2482         | 711          | 5’-TCACTGGCTCTGGTTCTG-3’ |
| IV      | R       | 700          | 789          | 5’-TCACTGGCTCTGGTTCTG-3’ |
| V       | R       | 1170         | 819          | 5’-TCACTGGCTCTGGTTCTG-3’ |
| VI, set 2| F   | 251          | 699          | 5’-TCACTGGCTCTGGTTCTG-3’ |
| VII     | F       | 1070         | 940          | 5’-TCACTGGCTCTGGTTCTG-3’ |
| VIII    | R       | 1991         | 599          | 5’-TCACTGGCTCTGGTTCTG-3’ |

* F, forward primer; R, reverse primer.

Synaptotagmin and Insulin Secretion

**RESULTS**

Identification of Synaptotagmin Isoforms in Islets and Insulin-secreting b-Cells—The expression of synaptotagmin mRNA (isoforms I through VIII) in pancreatic islet cells and insulin-secreting cell lines bTC3 and RINm5F was examined by RT-PCR with specific primers (Fig. 1). mRNAs of Syt isoform III (positive with both sets of primers), IV, V, and VII were found in pancreatic islet cells, whereas isoform I (positive with both sets of primers), II, III (positive with both sets of primers), IV, V, VII, and VIII were found in bTC3 cells. The rest of the
isofoms were not found in islets or βTC3 cells. Because the affinities for Ca\(^{2+}\) binding of isofoms III and VII are in the low micromolar range (23, 24), they were chosen for further investigation. It was also demonstrated that these two isofoms were present in RINm5F β-cells.

C-terminal peptides of III (CP3) and VII (CP7) isofoms were then synthesized and used as antigen to generate specific antibodies anti-Syt III and anti-Syt VII for protein studies (Fig. 2). Cell lysate of rat brain (indicated by B), pancreatic islets (I), βTC3 (β), and RINm5F (R) β-cells was prepared and analyzed by immunoblotting. The Syt III protein was present in rat brain with two different molecular masses of about 66 and 50 kDa. The Syt III protein of the same molecular mass was also present in rat islet (panel c), RINm5F (panels a and c), and βTC3 (panel a) cells with about equal amount of proteins in islet and RINm5F β-cells but less protein in βTC3 cells. The protein band of Syt III detected in this study was considered to have the expected molecular weight. 2) The anti-Syt III antibody binding was blocked by the specific peptide CP3, as shown by the comparison between the control in panel e and the dose-dependent (1–100 μg/10 ml) inhibition in panels f–i. 3) The band was not inhibited by a peptide of another isofom, 100 μg/10 ml CP7 (panel j). The Syt VII protein was also present in rat islet (panel d), RINm5F (panels b and d), and βTC3 (panel b) cells with about equal amounts of protein in islet, RINm5F, and βTC3 cells.

Subcellular Localization of Synaptotagmin III and VII in β-Cells—The subcellular localization of Syt III and VII in pancreatic islet cells and RINm5F β-cells was examined by confocal microscopy (Fig. 3). Insulin was stained in an intracellular vesicle pattern in RINm5F β-cells (red in row A). The staining of Syt III (green in row A) was found to have a similar pattern. Superimposition of the insulin and Syt III images indicated that the two proteins were co-localized in RINm5F β-cells. Similarly Syt VII was found to be co-localized with insulin in a vesicle pattern (row B). Such co-localization of Syt III (row C) and Syt VII (row D) with insulin was also demonstrated in primary rat pancreatic islet cells. These results indicate that Syt III and VII may have important roles in insulin secretion of β-cells.

**Insulin Secretion in Intact Stable β-Cells Overexpressing Synaptotagmin III**—In order to examine the functional role of Syt, overexpression of Syt III was achieved by stable transfection of Syt III in RINm5F β-cells (designated as Syt III β-cell). A 2.3-fold increase of Syt III protein level was demonstrated in the transfected cell line by Western blot (Fig. 4, panel A). Since RINm5F cells do not respond to glucose stimulation, the effects of other stimuli on insulin secretion were examined. In empty vector-transfected β-cells (Neo), 15 mM glyceraldehyde stimulated insulin secretion by about 2-fold, and the increase was about 2.4-fold in Syt III β-cells. However, the small difference between Neo and Syt III β-cells was not statistically significant (Fig. 4, panel B). Carbachol-induced insulin secretion in Neo (154 ± 16) and Syt III (177 ± 13) cells was not significantly different (>0.05). In contrast, 500 μM carbachol + 15 mM glyceraldehyde increased insulin secretion by about 3.5-fold in Syt III β-cell, which was significantly higher than the 2-fold increase in Neo β-cells (Fig. 4, panel C, p < 0.05). 50 mM potassium increased insulin secretion by about 30-fold in Syt III β-cell, which was significantly higher than the 6-fold increase obtained in Neo β-cells (Fig. 4, panel D, p < 0.05). These data show that increased cytosolic Ca\(^{2+}\) induced by carbachol or potassium is more effective for insulin exocytosis in β-cells overexpressing Syt III.

**Insulin Secretion in Permeabilized Stable β-Cells Overexpressing Synaptotagmin III**—This increased sensitivity of Syt III β-cells to Ca\(^{2+}\) was also demonstrated in permeabilized

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**Fig. 1. Synaptotagmin isoform expression in pancreatic islet and βTC3 and RINm5F β-cells.** Total RNA was extracted from isolated rat pancreatic islets (panel a) and βTC3 cells (panel b) and RINm5F β-cells (panel c). After DNase treatment of RNA, reverse transcription and polymerase chain reaction was performed with specific Syt primers (I–VIII). PCR products were analyzed on 2% agarose gel and identified with ethidium bromide staining. Representative results are from three experiments.

**Fig. 2. Western blot analysis of synaptotagmin III and VII isofoms in pancreatic islet and βTC3 and RINm5F β-cells.** C-terminal peptides of synaptotagmin III (CP3) and VII (CP7) isofoms were synthesized for generation of isoform-specific antibodies. Cell lysate of rat brain (indicated by B), pancreatic islets (I), βTC3 (β), and RINm5F (R) β-cells was prepared and analyzed by SDS-PAGE. Protein was transferred to cellulose membrane, blotted with anti-Syt III (panels a and c) or anti-Syt VII (panels b and d) antibodies, and subsequently with 125I-labeled protein-A. Protein bands were visualized and quantified using a PhosphorImager. In panels e–j, the primary antibody anti-Syt III was incubated in the absence of CP3 (panel e) or in the presence of 1, 10, 50, and 100 μg/10 ml CP3 (panels f–i), or 100 μg/10 ml CP7 (panel j). Figure is representative of at least three experiments.
Neo and Syt III β-cells were permeabilized by digitonin treatment. Intracellular Ca\(^2+\) level was then buffered to various levels with EGTA (Fig. 5). In Neo β-cells, increasing intracellular Ca\(^2+\) from 0 to 200 μM did not affect the rate of insulin secretion (100% versus 118 ± 27%, p > 0.05), whereas it increased insulin secretion significantly from 100% to 157 ± 20% in Syt III cells (p < 0.05). An increase of intracellular Ca\(^2+\) from 0 to 1000 μM also did not affect the rate of insulin secretion in Neo β-cells (100% versus 154 ± 42%, p > 0.05) but increased insulin secretion significantly from 100% to 231 ± 35% in Syt III β-cells (p < 0.05). When stimulated with 200 μM Ca\(^2+\) plus 10 μM mastoparan, the rate of insulin secretion was increased significantly from 100% to 180 ± 27% in Neo β-cells (p < 0.05), whereas it was increased significantly from 100% to 295 ± 31% in Syt III β-cells (p < 0.05). Thus, mastoparan-induced insulin secretion in Syt III was higher than in control Neo β-cells. Similarly higher insulin secretion in Syt III β-cells was also observed when 1000 μM Ca\(^2+\) plus 10 μM mastoparan was used. In another set of experiment, cells were permeabilized by streptolysin O treatment. Insulin secretion in the presence of 10 μM intracellular Ca\(^2+\) was about 3.5-fold that of the basal secretion with 0.1 μM Ca\(^2+\) in Syt III cells. This stimulation was significantly more potent than the 2-fold increase in Neo β-cells (p < 0.05). GTPγS-induced insulin secretion in streptolysin O-permeabilized cells was the same between control and overexpressing cells (Fig. 6).

**Insulin Secretion in β-Cells Overexpressing Synaptotagmin VII**—In contrast to the lack of amplification in Syt III cells, carbachol-induced insulin secretion in intact Syt VII cell (354 ± 86%) was amplified compared with Neo cells (154 ± 16%) as shown in Table II. The effect of glyceroldehyde in Syt VII cell (336 ± 35%) was greater than those in Syt III (242 ± 20%) or Neo cells (198 ± 17%). The combination of carbachol and glyceroldehyde caused a greater effect in Syt VII (468 ± 59%) than in Syt III (340 ± 33%) or Neo (203 ± 24%) cells. The effects of mastoparan or 30 mM K\(^+\) were the same between Syt VII and Neo cells. In permeabilized cells, the effects of 200 or 1000 μM Ca\(^2+\) were significantly higher in Syt VII than in Neo cells. Mastoparan plus high intracellular Ca\(^2+\) (either 200 or 1000 μM) also caused more secretion in Syt VII cells than in Neo cells.

**DISCUSSION**

Increased cytosolic Ca\(^2+\) is required for secretagogue-induced insulin secretion from pancreatic β-cells. The various isoforms of synaptotagmins are known to play major roles in regulated secretion of neurotransmitters in presynaptic terminals (22, 23). Synaptotagmin has been shown to bind Ca\(^2+\) in a phospholipid-dependent manner and undergoes a Ca\(^2+\)-dependent conformational change that enables it to bind syntaxin on the plasma membrane for granule fusion. The expression of Syt I and II mRNA and protein in pancreatic β-cells has recently been shown in insulin-secreting β-cell lines RINm5F, INS-1, and HIT-T15 (27). An involvement of Syt I or II in
insulin secretion of tumor cell lines is supported by the inhibition of Ca\(^{2+}\)-induced insulin secretion in permeabilized cells using antibodies directed against the Ca\(^{2+}\)-dependent phospholipid-binding site of the first C\(_2\) domain of Syt I or II. However, overexpression of Syt II in HIT-T15 \(\beta\)-cell failed to affect insulin secretion (27). Moreover, this hypothesis may not be applicable to normal pancreatic \(\beta\)-cells, because of the lack of Syt I and II expression in primary islet \(\beta\)-cell as shown previously and in this study (27). These observations suggest that Syt I and II do not have a physiological role in insulin secretion and imply the presence of alternative signaling Syt isoform expression between tumor cell line and primary rat islet cell. Another argument against the role of Syt I in insulin secretion is that it requires levels of free Ca\(^{2+}\) that are at least 1 to 2 orders of magnitude higher than those observed during insulin exocytosis in the \(\beta\)-cell (22, 45, 46).

Our study has discovered new Syt isoforms (IV, V, VII, and VIII) as well as confirmed previously reported Syt isoform (I, II and III) expression in \(\beta\)TC3 (I, II, III, IV, V, VII, and VIII), RINm5F cells (III and VII), and rat primary islet \(\beta\)-cells (III, IV, V, and VII). The expression of Syt VII protein in \(\beta\)TC3, RIMm5F, and primary rat islet is a novel finding. The absence of RT-PCR product for synaptotagmin isoforms I, II, VI, and VIII in primary rat islets suggests they are either not expressed or expressed at very low levels. The difference of isoform expression between tumor cell line and primary rat islet cell cannot be easily explained. However, this may indicate that isoforms III and VII are more physiologically important than isoforms I and II in insulin secretion. Further investigation is required to address the roles of Syt IV and V in pancreatic \(\beta\)-cell.

An important candidate for insulin exocytosis is the Syt III isoform (47, 48). Its Ca\(^{2+}\) sensitivity is submicromolar, which is the physiological range present in stimulated \(\beta\)-cells. Previous studies have mainly focused on Syt III identification in \(\beta\)-cells. Syt III has been found in the insulin-secreting cell line MIN6 as well as in primary islet cells (48). Syt III and VII have been localized to insulin granules in various \(\beta\)-cell lines and islet cells, which implicates that they may play important roles in granule fusion and exocytosis (47). Our study shows that by confocal microscopy, synaptotagmin III and VII co-localize with insulin secretory granules. Thus, all studies so far have localized Syt III in the \(\beta\)-cell and in particular to the insulin-containing secretory granule.
Because of the localization of Syt III and Syt VII to the insulin secretory granule, it is conceivable that they may have a major role in insulin exocytosis, by analogy with their known role in neurotransmitter release. Treatment of permeabilized MIN6 cells with anti-Syt III antibody inhibited Ca$^{2+}$-triggered insulin secretion (48). Another study reported that synaptotagmin III cells overexpressing synaptotagmin III. Control Neo β-cells and Syt III overexpressing β-cells were plated in 6-well dishes. β-Cells were permeabilized with streptolysin O as described under "Experimental Procedures." Permeabilized cells were washed, preincubated, and incubated in 1 ml of intracellular buffer with 0.1 M (Low Ca or control, open bars) or 10 μM Ca$^{2+}$ (High Ca, solid bars, left panel) or 100 μM GTP-β-S (solid bars, right panel). Results are shown as the mean ± S.E. of insulin secretion expressed as a percent of control (no secretagogues, open bars) from 12 observations per condition.

**TABLE II**

Effects of various conditions on insulin secretion of Neo and Syt VII cells

| Condition       | Neo β-cell | Syt VII β-cell | p     |
|-----------------|------------|----------------|-------|
| Intact cells    |            |                |       |
| Control         | 100        | 100            |       |
| +CCH            | 154 ± 16   | 354 ± 86       | <0.05 |
| +Gly            | 198 ± 17   | 336 ± 35       | <0.05 |
| +CCH+Gly        | 203 ± 24   | 468 ± 59       | <0.05 |
| +MP             | 158 ± 20   | 155 ± 13       |       |
| +K90            | 561 ± 88   | 631 ± 61       |       |
| Permeabilized cells |        |                |       |
| Ca0             | 100        | 100            |       |
| Ca0+MP          | 244 ± 40   | 195 ± 17       |       |
| Ca0.2           | 117 ± 27   | 234 ± 48       | <0.05 |
| Ca0+2-MP        | 179 ± 21   | 261 ± 27       | <0.05 |
| Ca1             | 154 ± 41   | 368 ± 100      | <0.05 |
| Ca1+MP          | 195 ± 25   | 538 ± 113      | <0.05 |

Our results clearly show that β-cells overexpressing Syt III secrete more insulin in response to stimuli that increase intracellular Ca$^{2+}$. This effect was consistently observed in intact β-cells as well as permeabilized β-cells. In normal β-cells, depolarization with K$^+$ causes an influx of extracellular Ca$^{2+}$ and subsequent insulin secretion. However, in the β-cells overexpressing Syt III, K$^+$-induced insulin secretion was further amplified 6-fold. In the case of glyceraldehyde- and carbachol-induced insulin secretion, the amplification obtained was only 2.5-fold. This is probably due to the fact that cytosolic Ca$^{2+}$ elevation is the sole mechanism for K$^+$-induced insulin secretion, whereas glyceraldehyde and carbachol stimulate insulin secretion through increased intracellular Ca$^{2+}$ as well as other mechanisms, such as activation of protein kinase C (8–17). Similarly, the lack of amplification of glyceraldehyde-induced secretion in Syt III β-cells is probably due to the even lower contribution of Ca$^{2+}$-dependent signaling in this pathway. Increased Ca$^{2+}$-induced insulin secretion was also directly observed in permeabilized Syt III β-cells. Mastoparan, which directly triggers insulin granule fusion and exocytosis (33), also increased insulin secretion. The findings in our study suggest that Syt III and Syt VII may be the Ca$^{2+}$ sensor or one of the Ca$^{2+}$ sensors for insulin exocytosis of the β-cell and may be a potentially interesting target for cellular and pharmacological therapies aimed at increasing insulin secretion. The possibility that Syt IV or V isoforms play a role in Ca$^{2+}$ sensing for insulin exocytosis cannot be excluded.

It is interesting that both Syt III and Syt VII β-cells had increased insulin secretion induced by direct elevation of intracellular Ca$^{2+}$ concentration in permeabilized cells. However, they secreted insulin differently in response to carbachol and high K$. The amplified response to 30 mM K$^+$ was larger in Syt III cells and not significant in Syt VII cells. On the other hand, insulin secretion triggered by carbachol was larger in Syt VII cells but was not significant in Syt III cells. The explanation of these differences is currently unclear. One possibility is that the two Syt isoforms sense changes of cytosolic Ca$^{2+}$ in different subcellular locations. For example, Syt III may sense an increase in intracellular Ca$^{2+}$ due to influx of extracellular Ca$^{2+}$, whereas Syt VII may be relevant when intracellular Ca$^{2+}$ elevation is induced by intracellular mobilization through inositol 1,4,5-trisphosphate receptors. It has been demonstrated that synaptotagmin directly interacts with N-type (49–57) or P/Q-type (19, 54, 55) Ca$^{2+}$ channels in neurons and Le-type Ca$^{2+}$ channels (20). Inositol 1,4,5-trisphosphate receptors are present in pancreatic β-cells; however, it is currently unclear whether they associate with synaptotagmin.

In conclusion, we have shown that isoforms III, IV, V, and VII of synaptotagmin instead of isoforms I and II are expressed in islet β-cells and that Syt III and Syt VII may have an important physiological role in insulin exocytosis of the pancreatic β-cell. The physiological roles of the other isoforms remain to be investigated.

**Acknowledgment—**The Diabetes Endocrinology Research Center Radioimmunoassay Core and the Biomedical Imaging Core are supported by National Institutes of Health Grant DK19525.

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