Syntaxin, but not soluble NSF attachment protein (SNAP), biosynthesis by rat pancreatic islets is regulated by glucose in parallel with proinsulin biosynthesis

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Summary Recent studies have revealed that soluble N-ethylmaleimide sensitive factor attachment receptor (SNARE)-related proteins, originally identified in neural tissues, are also expressed in pancreatic beta cells. In this study, we investigated the effect of glucose on syntaxin 1 and α/β SNAP biosynthesis in pancreatic beta cells and we demonstrated that syntaxin 1, but not α/β SNAP biosynthesis by rat isolated pancreatic islets was stimulated specifically by glucose nearly in parallel with proinsulin biosynthesis. Stimulation of syntaxin 1 and proinsulin biosynthesis by glucose was dose-dependent ($K_m = \sim 8$ mmol/l) and reached the maximum (about 8–12 fold) at concentrations over 11 mmol/l. In contrast, 22 mmol/l glucose increased α/β SNAP biosynthesis about 2-fold only, similar to the increase in

total protein synthesis. Stimulation of syntaxin 1 biosynthesis by glucose was also time-dependent, taking around 3 h to reach the maximum, and was not affected by actinomycin-D, suggesting regulation at the translational level. On the other hand, glucose had a similar stimulating effect on both syntaxin 1 and α/β SNAP biosynthesis by mouse insulinoma β TC3 cells as it did on proinsulin biosynthesis. The evidence showing coordinated stimulation of syntaxin 1 and proinsulin biosynthesis by glucose in rat islets suggested the critical functional role of syntaxin 1 in the insulin exocytotic mechanism. [Diabetologia (1997) 40: 1396–1402]

Keywords Syntaxin, SNAP, insulin biosynthesis, SNARE, glucose.

The process of insulin exocytosis is thought to be divided fundamentally into two stages. The first involves glucose transportation into the cells, where it is metabolized and the metabolite increases the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). During the later stage, the secretory vesicles move to the plasma membrane, where they dock, fuse and release their contents into the extracellular space as a result of the elevated $[\text{Ca}^{2+}]_i$. Recent advances in brain research have

identified the basic components, such as synaptic vesicle-associated membrane proteins (v-SNAREs) and their cognate target membrane proteins (t-SNAREs), of the machinery that control the targeting and fusion of synaptic vesicles with plasma membranes during the processes involved in the later exocytotic stage [1, 2]. Characterization of these proteins and subsequent studies of their properties have led to a model called the SNARE hypothesis that explains vesicle docking and fusion [2]. Recently, several workers have shown that pancreatic beta cells, as well as neurons, express N-ethylmaleimide sensitive factor (NSF), α/β soluble NSF attachment proteins (α/β SNAP) and SNAP receptors (SNAREs) such as synaptotagmin, syntaxin and SNAP-25 [3–6]. Therefore, the SNARE hypothesis [7, 8], which was proposed for the neurotransmitter release mechanism, may be applicable to insulin exocytosis in pancreatic beta cells, even though they are not identical to neurons.

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Abbreviations: NSF, N-ethylmaleimide sensitive factor; SNAP, soluble NSF attachment protein; SNARE, SNAP receptor; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate buffered saline.

Insulin biosynthesis and release by pancreatic beta cells in the islets of Langerhans are regulated by glucose [9, 10], and stimulation of insulin release by glucose may increase the demand for α/β SNAP, NSF and SNAREs. For example, syntaxin 1A may be recycled by plasma membranes and insulin secretory vesicles which are known to be dense core granules, as shown in chromaffin cells by Tagaya et al. [11], although there is no clear evidence of syntaxin 1A recycling in pancreatic beta cells. Furthermore, α/β SNAP may be a rate-limiting factor for insulin exocytosis as in neurotransmitter release [12–15]. Therefore, an increased demand for these proteins may be compensated for by parallel stimulation of the biosynthesis of syntaxin 1A and α/β SNAP involved in glucose-stimulated insulin exocytosis. In this study, we investigated whether or not glucose regulates syntaxin 1 and α/β SNAP biosynthesis by rat isolated pancreatic islets and the insulinoma cell line, β TC3.

Materials and methods

Materials. Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 and fetal bovine serum (FBS) were obtained from GIBCO BRL (Gaithersburg, USA). RPMI 1640 medium with different glucose concentrations was prepared using the selected amine kit from GIBCO BRL. Amplify was from Amersham Corporation (Amersham, Bucks., UK), and the protein labelling mix EXPRE³⁵SS (³⁵S-methionine/cysteine) was purchased from Dupont NEN (New England Nuclear, Boston, Mass., USA). Protein A-Sepharose was from Pharmacia (Piscataway, N.J., USA). Actinomycin D was from Sigma (St. Louis, Mo., USA).

Islet isolation and culture of β TC 3 cells. Pancreatic islets from male, 200 g, Wistar rats were isolated by collagenase digestion, as described previously [16], and picked up under a dissecting microscope. For the experiments with different glucose concentrations, the islets were incubated in PRMI 1640 medium containing 10 % (v/v) dialysed FBS and the required glucose concentration for the required time under an atmosphere of 5 % CO₂ and 95 % O₂ at 37 °C. The β TC3 cells, a gift from Dr. D. Hanahan (University of California, San Francisco, Calif., USA), were cultured and maintained in DMEM, as described previously [17].

Antibodies. Mouse monoclonal antibody against rat syntaxin 1A/Hpc-1 was obtained from Sigma. In order to study the specificity of this antibody, recombinant syntaxin 1a protein was produced as described previously [18]. The affinity-purified anti- α/β -SNAP antiserum produced in rabbits using the α/β -SNAP synthetic peptide was a generous gift from Dr. M. Takahashi (Mitsubishi Kasei Institute of Life Sciences, Tokyo, Japan). The antiserum against this peptide reacted with both recombinant bovine α - and mouse β -SNAP proteins produced by *Escherichia coli* M15 (data not shown).

Metabolic labelling. For metabolic labelling, unless otherwise stated, batches of 100–200 islets or $0.5\text{--}1 \times 10^6$ β TC3 cells were labelled in a 0.2-ml reaction mixture comprising methionine/cysteine/leucine-free RPMI 1640 containing 10 % (v/v) dialysed FBS and the required glucose concentration with

100 μ Ci each of [³⁵S] methionine/cysteine and [³H] leucine for 3 h at 37 °C. After labelling, the islets or β TC3 cells were centrifuged briefly at 10000 rev/min, the labelling medium was removed and the pellets were washed once with ice-cold phosphate buffered saline (PBS).

Immunoprecipitation. Labelled cell pellets were extracted by sonication in 0.1 ml of TAS buffer (0.1 mol/l Tris-HCl, 0.05 mol/l NaCl, 0.25 % (w/v) bovine serum albumin, 0.1 % (v/v) Triton X-100, pH 7.5) containing a cocktail of protease inhibitors (1 mmol/l phenylmethylsulfonyl fluoride, 50 μ g/ml trypsin, 10 μ g/ml leupeptin, and 5 μ g/ml pepstatin A). Nuclei and insoluble debris were pelleted by centrifugation for 5 min in a microcentrifuge and the supernatant (total cell lysate) was collected, preadsorbed with preimmune rabbit or mouse serum by incubation with protein A-Sepharose beads for 2 h at 4 °C and then subjected to immunoprecipitation with protein A-Sepharose beads with the required antiserum for 24 h at 4 °C. The resulting Sepharose pellets were washed twice with ice-cold wash buffer (0.6 mol/l NaCl, 10 mmol/l Tris (pH 8.6), 0.05 % Nonidet P-40 and 0.1 % sodium dodecyl sulfate (SDS)) and once with ice-cold TS buffer (0.1 mol/l Tris-HCl, 0.05 mol/l NaCl, pH 7.6). The pellets were eluted from the Sepharose by boiling with reducing SDS sample buffer and analysed by one-dimensional SDS electrophoresis on a 12 % polyacrylamide gel. After fixation, the gels were prepared for fluorography by treatment with Amplify, dried, autoradiographed and the band intensities were quantified by laser scanning densitometry (Image Master version 1.20; Pharmacia Biotech).

Immunoblot analysis. For immunoblotting, 200 islets were lysed as described above, the proteins were separated by electrophoresis and transferred to nitrocellulose membrane filters in a blotting buffer consisting of 25 mmol/l Tris, 190 mmol/l glycine and 20 % (v/v) methanol. Non-specific antibody binding was blocked by incubating each filter with 5 % non-fat dried milk in PBS. Next the filters were incubated with the required primary antibody (1:500–1000 dilution) for 4 h, washed twice with 0.05 % Nonidet P-40 and 0.1 % Tween 20 in PBS, then incubated with horseradish peroxidase-conjugated secondary antibody for 1 h. Finally, the bands were visualized using a chemiluminescence detection system (NEN).

Insulin biosynthesis and total protein synthesis. Prior to immunoprecipitation, 10 μ l aliquots were taken from each cell lysate for insulin immunoprecipitation using a guinea pig anti-insulin antibody (DAKO, Glostrup, Denmark), as described elsewhere [17]. Total protein synthesis was determined by precipitating the cell lysates with 10 % (v/v) trichloroacetic acid, solubilizing the resulting pellets in 3 mol/l acetic acid and counting their radioactivity using a liquid scintillation counter.

Northern blot analysis. We subjected 30 μ g of the total RNA isolated from mouse β TC3 cells to a 1.5 % agarose, 0.66 mol/l formaldehyde gel, blotted onto nitrocellulose filters, and hybridized with ³²P-labelled mouse syntaxin 1A cDNA probe. The filters were washed (0.1 $\times$ standard sodium citrate, 0.1 % SDS, 55 °C), and exposed to Kodak X-Omat AR film at –80 °C.

Statistical analysis. Data are presented as means $\pm$ SEM. The significance of differences between groups was determined by analysis of variance (ANOVA) and those at $p < 0.05$ were considered significant.

Results

In experiments designed to investigate the effects of glucose on syntaxin 1, α/β SNAP and proinsulin biosynthesis, rat isolated islets were radiolabelled for 3 h. In order to analyse syntaxin 1 and α/β SNAP protein biosynthesis, lysates of labelled islets were immunoprecipitated with anti-syntaxin 1 and anti- α/β -SNAP antibodies, then subjected to SDS-polyacrylamide gel electrophoresis (PAGE) followed by autoradiography. Before analysing the biosynthesis study, we checked the specificities of antibodies and performed the antibody titration study. Figure 1 demonstrated the specificities of these antibodies. They reacted with syntaxin 1 and α/β SNAP proteins, respectively, and the addition of an excess amount of recombinant proteins completely displaced the endogenous proteins. We deduced that the syntaxin 1 protein band which migrated to about 35 kDa was syntaxin 1A, because we previously demonstrated that only syntaxin 1A, but not syntaxin 1B can be amplified by RT-PCR [18]. The molecular mass of α/β SNAP was approximately 35 kDa in our gel analysis. Although the molecular masses of α - and β -SNAP in neuronal tissues were reported to be 33 and 34 kDa, respectively [12, 19], our gel analysis did not enable us to establish whether this protein band was α - or β -SNAP or a combination of both. In order to establish the ability of antibodies to precipitate the syntaxin 1 and α/β SNAP proteins by immunoprecipitation method, 10 ng and 1 μ g of syntaxin 1 and α -SNAP recombinant proteins were immunoprecipitated with anti-syntaxin 1 and anti- α/β -SNAP antibodies, respectively. Immunoprecipitants were analysed by immunoblot analysis with corresponding antibodies. Both antibodies were able to precipitate at least 1 μ g of recombinant proteins (Fig. 2). Although the data are not shown, immunoblot analysis of supernatants did not show any detectable band of either protein, indicating that all recombinant protein was precipitated. Since immunoblot analysis of cell lysates of 200 islets showed only weakly stained bands of syntaxin 1 and α/β SNAP (Fig. 2), these antibodies were confirmed to precipitate all endogenous proteins of syntaxin 1 and α/β -SNAP in islets by an immunoprecipitation method.

Glucose stimulated syntaxin 1A biosynthesis specifically in a manner similar to its stimulation of proinsulin (Fig. 3). Stimulation was detected with glucose in excess of 5.5 mmol/l and reached the maximum at concentrations over 11 mmol/l and the maximal stimulation of syntaxin 1 biosynthesis was about 8-fold. In contrast, α/β SNAP biosynthesis was affected little over the range of glucose concentrations used (only a 2–3-fold increase). The glucose-stimulated biosynthesis of α/β SNAP followed that of total islet protein (about a 2-fold increase). Next, we investigated the effects of actinomycin D, an inhibitor of mRNA

Fig. 1. Immunoprecipitation analysis of syntaxin 1 and SNAP proteins in rat isolated islets. We isolated 200 rat islets by collagenase digestion and radiolabelled for 3 h with ^{35}S -Met/Cys and ^3H -leucine in RPMI 1640 containing 10% (v/v) dialysed FBS in the presence of 5.5 mmol/l glucose. The islets were disrupted, immunoprecipitated with anti-syntaxin 1 and anti-SNAP antibodies in the presence and absence of 10 μ g of recombinant proteins, as described in Materials and methods, and the immunoprecipitates were analysed using 12% SDS-PAGE, followed by fluorography with Amplify and autoradiography

Fig. 2A, B. Titration study of antibodies and immunoblot analysis of syntaxin 1 and α/β SNAP in rat islets. **A)** Titration study: 10 ng and 1 μ g of recombinant syntaxin 1 and α SNAP proteins, produced by *E. coli* and purified, were immunoprecipitated with anti-syntaxin 1 and anti- α/β -SNAP antibodies, respectively. The pellets were dissolved in reducing SDS sample buffer and analysed by 12% SDS-PAGE, then transferred to a nitrocellulose filter. The filter was reacted with corresponding antibodies and the band was visualized using a chemiluminescence detection system. **B)** Immunoblot analysis of rat islets: Total cell lysates from 200 islets were subjected to immunoblot analysis, as described in Materials and methods, using specific anti-syntaxin 1 and anti- α/β -SNAP antisera

synthesis [20]. Radiolabelling of rat isolated islets for 3 h in the presence of actinomycin D affected neither glucose-stimulated syntaxin 1 nor proinsulin biosynthesis (Table 1), indicating that glucose regulation was predominantly at the translational level. The stimulation of both syntaxin 1 and proinsulin biosynthesis by glucose was time-dependent (Fig. 4). Significant stimulation of proinsulin biosynthesis was detected within 20 min of preincubation, whereas that of syntaxin 1 was not detected until after 2 h of preincubation and increased with time thereafter, reaching a steady-stimulated-state after 3 h of preincubation (Fig. 4). Thus, the patterns of syntaxin 1 and proinsulin biosynthesis differed over time, but the effects of glucose were specific and their patterns were not identical to that of total protein synthesis.

We also investigated whether glucose regulates syntaxin 1 and α/β SNAP biosynthesis by the mouse

Fig. 3. Dose-dependent stimulation of proinsulin, syntaxin 1 and α/β SNAP biosynthesis by glucose. Rat isolated islets were incubated for 3 h with the indicated glucose concentrations in the presence of ^{35}S -Met/Cys and ^3H -leucine. Proinsulin and total protein synthesis were measured, as described in Materials and methods. Syntaxin 1 and α/β SNAP were immunoprecipitated from islet lysates with specific antisera and analysed by 12 % SDS-PAGE followed by fluorography and densitometric analysis. The results are expressed as arbitrary scan units, relative to values obtained in the presence of basal (2.2 mmol/l) glucose. The means of five separate experiments are shown. Insert shows a typical autoradiogram of syntaxin 1 and α/β SNAP protein analysed by immunoprecipitation

insulinoma cell line βTC3 , by which insulin secretion and biosynthesis were found to respond to glucose after a long incubation period [17]. After preincubation for 3 or 24 h with either 0 or 11 mmol/l glucose, βTC3 cells were radiolabelled for 3 h in the presence of the same glucose concentration. Syntaxin 1 and α/β SNAP biosynthesis after preincubation for 3 h in the presence or absence of 11 mmol/l glucose did not differ, but the biosynthetic rates of cells preincubated with 11 mmol/l glucose for 24 h were about 3 times higher than those of cells preincubated without glucose (Table 2). In fact, Northern blot analysis with rat syntaxin 1A cDNA probe showed that the level of syntaxin 1A mRNA in cells treated with a high

Fig. 4. Time courses of glucose-stimulated proinsulin and syntaxin 1 biosynthesis. Rat isolated islets were maintained in the presence of 2.2 mmol/l glucose for 1 h, then preincubated for the indicated times with 22 mmol/l glucose, after which, they were labelled with ^{35}S -Met/Cys and ^3H -leucine for 45 min in the presence of 22 mmol/l glucose. Proinsulin and syntaxin 1 were immunoprecipitated with specific antisera and analysed as described in Materials and methods. The results are expressed as values relative to those obtained at the start of the preincubation period (0 min). The means of 3 separate experiments are shown

glucose concentration increased compared to that in non-glucose-treated cells only after a 24 h preincubation period (Fig. 5). The pattern of glucose-stimulated proinsulin biosynthesis by βTC3 cells was similar to the syntaxin 1 and α/β SNAP biosynthetic patterns. Accordingly, syntaxin 1 and α/β SNAP biosynthesis by βTC3 cells was regulated by glucose in a similar manner to that observed in proinsulin biosynthesis.

Discussion

Recently, it was demonstrated that syntaxin 1 [18, 21] and α -SNAP [22] perform specialized roles in insulin secretion, indicating that these proteins must be synthesized and assembled in a co-ordinated manner to ensure integrated functioning of the organelle. As

Table 1. Effect of actinomycin D on glucose-stimulated proinsulin, syntaxin 1, and α/β SNAP biosynthesis

	Increase (-fold) relative to 2.2 mmol/l glucose			
	Proinsulin	Syntaxin 1	α/β SNAP	Total protein
-Actinomycin D	11.5 ± 1.5	8.6 ± 1.8	3.1 ± 1.1	2.8 ± 0.5
Actinomycin D (10 $\mu\text{mol/l}$)	10.3 ± 2.3	7.0 ± 1.3	2.6 ± 0.8	2.1 ± 0.6^a

Isolated rat islets were incubated for 3 h in 22 or 2.2 mmol/l glucose $\pm$ actinomycin D with ^{35}S -Met-Cys and ^3H -Leucine. Proinsulin, syntaxin 1, and α/β SNAP were immunoprecipitated from islet lysates as described in Materials and methods. Total protein synthesis was determined by trichloroacetic acid

(10 % (v/v)) precipitation. Results (mean $\pm$ SEM for 3–5 experiments) are expressed relative to values obtained at a basal 2.2 mmol/l glucose

^a $p < 0.05$ vs actinomycin D

Table 2. Glucose regulation of proinsulin, syntaxin 1 and α/β SNAP biosynthesis by β TC3 cells

Incubation period	Increase (-fold) relative to 0 mmol/l glucose			
	Proinsulin	Syntaxin 1	α/β SNAP	Total protein
3 h	1.1 $\pm$ 0.5	0.9 $\pm$ 0.3	1.4 $\pm$ 0.1	1.2 $\pm$ 0.3
24 h	2.1 $\pm$ 0.3	3.3 $\pm$ 0.8	3.4 $\pm$ 1.1	1.9 $\pm$ 0.5

β TC3 cells were incubated for 3 or 24 h in RPMI 1640 medium containing $\pm$ 11 mmol/l glucose. After incubation, the medium was removed and cells were then labelled for 3 h with [35 S] Met-Cys and [3 H] Leucine in the presence of the same glucose concentration as that used before incubation. Proinsulin, syntaxin 1, and α/β SNAP were immunoprecipitated from total cell lysates as described in Materials and methods. Results (mean $\pm$ SEM for 3 experiments) are expressed as relative to values obtained at 0 mmol/l glucose

insulin biosynthesis is regulated by glucose predominantly at the level of preproinsulin mRNA translation [9, 10], the biosynthetic control exerted by glucose appears to extend to these proteins. At present, it is not known whether these proteins respond to glucose in a similar manner to proinsulin.

In this study, we investigated the effects of glucose on syntaxin 1 and α/β SNAP biosynthesis by radiolabelled rat islets and β TC3 cells using immunoprecipitation analysis. Our results indicated that syntaxin 1, but not α/β SNAP biosynthesis is regulated in coordination with proinsulin biosynthesis by rat isolated islets. Glucose stimulated syntaxin 1 biosynthesis in a manner similar to its stimulation of proinsulin biosynthesis and glucose regulation of syntaxin 1 mimicked that of proinsulin biosynthesis. The magnitudes of the glucose-stimulated syntaxin 1 and proinsulin biosynthetic responses were almost the same, whereas those of the glucose-stimulated α/β SNAP and total protein synthetic responses were almost identical. Although it is conceivable that glucose directly stimulated syntaxin 1 biosynthesis, it may increase the immunogenicity of syntaxin 1 to the antibody instead of direct biosynthetic stimulation, since this protein is known to be part of a multiprotein complex [23–25]. Northern blot analysis with syntaxin 1 cDNA probe may provide an answer to this question. However, Northern blot data by rat islets did not show an appreciable amount of syntaxin 1 mRNA because of its extremely low level of expression in islets (data not shown). Although syntaxin 1 biosynthesis required a relatively long time (about 3 h) to reach a steady state, incubation with actinomycin D did not affect its rate, suggesting that translational control was operational. Indeed, the specific regulation of proinsulin biosynthesis by glucose occurred predominantly at a translational level [9, 26]. Biosynthesis of several secretory granule proteins [27], as well as PC3 endopeptidase [28, 29], is also stimulated specifically by glucose at a translational level. One possible mechanism involves an increase in the rate of signal recognition

Fig. 5. Northern blot analysis of syntaxin 1 A mRNA in mouse β TC3 cells. After β TC3 cells were treated with and/or without 11 mmol/l glucose for 3 or 24 h, total cellular RNAs were isolated and 30 μ g samples were electrophoresed, transferred to a nitrocellulose filter, and hybridized with 32 P-labelled syntaxin 1 cDNA probe. The autoradiogram was exposed for 4 days

particle-mediated translocation of nascent peptides into the endoplasmic reticulum lumen, as proposed for insulin synthesis [26]. In agreement with this hypothesis, glucose did not stimulate α/β SNAP biosynthesis selectively, as this cytoplasmic protein is presumably synthesized on free ribosomes. However, this hypothesis does not completely fit the mechanism responsible for glucose-regulated syntaxin 1 biosynthesis, because the membrane-spanning region of syntaxin 1 is localized at the extreme COOH-terminal [30, 31], indicating that synthesis would be completed by the ribosomes before the transmembrane region was recognized by signal recognition particle. Syntaxin 1 may be inserted post-translationally into endoplasmic reticulum membranes [32], by the interaction with signal recognition particles or other molecules. Indeed, Jantti et al. [33] indicated that yeast syntaxin Sso2p predominantly uses a post-translational endoplasmic reticulum insertion mechanism, but whether the insertion process involves signal recognition particles, or some other, yet unidentified protein translocation machinery, remains to be determined. Thus, the mechanisms responsible for glucose-regulated syntaxin 1 and insulin biosynthesis may not be the same and the different time courses of glucose-regulated syntaxin 1 and proinsulin biosynthesis shown in Figure 4 appear to represent the different mechanisms, however the mechanism remains largely obscure.

On the other hand, the data yielded by the insulinoma cell line β TC3 experiments showed that glucose stimulated both syntaxin 1 and α/β SNAP biosynthesis to almost the same extent. Glucose stimulated the biosynthesis of these proteins only after a 24-h preincubation period, similar to its stimulation of proinsulin biosynthesis. As demonstrated previously [17], glucose only regulates proinsulin biosynthesis by insulinoma β TC3 cells at the transcriptional

level, because these cells have lost selectively the translational control of insulin biosynthesis, as have RINm5F cells [34] and it is noteworthy that the regulation pattern of proinsulin biosynthesis by β TC3 cells followed those of syntaxin 1 and α/β SNAP biosynthesis. Indeed, the data of Northern blot analysis of syntaxin 1A mRNA indicated that glucose increased syntaxin 1A biosynthesis at the transcriptional level by a mechanism similar to that of proinsulin biosynthesis. The reason why α/β SNAP biosynthesis is regulated by glucose in parallel to that of proinsulin only in β TC3 cells is not known at present. As mentioned above, insulin biosynthesis in β TC3 cells responded only after prolonged exposure to glucose, and these cells do not have a secretory pathway regulated by the physiological concentration of glucose observed in intact islets. Accordingly, the different glucose-regulated process of syntaxin/ α/β SNAP/proinsulin observed in β TC3 cells appears to represent a marked characteristic difference between insulinoma cell line and primary islet cells.

It is of interest that glucose stimulated syntaxin 1 biosynthesis by rat islets and β TC3 cells, because syntaxin 1A overproduction by β TC3 cells inhibited glucose-stimulated insulin release [18]. In view of its function as a negative regulator, glucose would be expected to regulate syntaxin 1 biosynthesis negatively. However, we would like to emphasize that syntaxin 1A functions in the insulin exocytotic pathway, whether as a negative or positive regulator, and must be an important part of the insulin exocytotic machinery. Therefore, it would not be surprising if glucose did upregulate syntaxin 1 biosynthesis. Indeed, treatment of rat islets with an anti-syntaxin 1 antibody inhibited, rather than stimulated, insulin release [21]. Although the physiological significance of glucose-stimulated syntaxin 1 biosynthesis in rat islets is still to be determined, it is conceivable that glucose-stimulated the syntaxin 1 biosynthesis in order to compensate for the deficit of this protein in glucose-stimulated insulin exocytosis if syntaxin 1 plays a role in the storage of secretory granules as proposed by Bittner et al. [35]. On the other hand, as α/β SNAP protein was shown to stimulate hormone release from permeabilized adrenal chromaffin [12] and insulin secreting [22] cells, stimulation of α/β SNAP biosynthesis by glucose is easier to understand. However, as shown above, glucose did not stimulate α/β SNAP biosynthesis by rat islets selectively in the short term incubation, probably because α/β SNAP protein in the cytoplasm is stable and rapid production may not be necessary.

In conclusion, glucose selectively stimulated syntaxin 1A and proinsulin biosynthesis in parallel by rat islets. This may be key element of the insulin exocytotic machinery that enable pancreatic beta cells to maintain constant levels of the proteins that comprise the exocytotic machinery.

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