Disrupted dynamics of F-actin and insulin granule fusion in INS-1 832/13 beta-cells exposed to glucotoxicity: partial restoration by glucagon-like peptide 1

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A B S T R A C T

Actin dynamics in pancreatic β-cells is involved in insulin exocytosis but the molecular mechanisms of this dynamics and its role in biphasic insulin secretion in pancreatic β-cells is largely unknown. Moreover, the impact of a glucotoxic environment on the sub-cortical actin network dynamics is poorly studied. In this study, we investigate the behavior of insulin granules and the subcortical actin network dynamics in INS-1 832/13 β-cells submitted to a normal or glucotoxic environment. Our results show that glucose stimulation leads to a re-organization of the subcortical actin network with a rupture of its interactions with t-SNARE proteins (Syntaxin 1A and SNAP-25), promoting insulin secretion in INS-1 832/13 β-cells. Prolonged exposure of INS-1 832/13 β-cells to high-glucose levels (glucotoxicity) leads to the densification of the cortical actin network, which prevents its reorganization under acute glucose, and diminishes the glucose-stimulated insulin secretion, as shown by the decreased number of fusion events. The most interesting in our results is the partial restoration by GLP-1 of the insulin secretion ability from high-glucose treated INS-1 832/13 cells. This improved insulin exocytosis is associated with partial restored actin dynamics and fusion events during the two phases of the secretion, with a preferential involvement of Epac2 signaling in the first phase and a rather involvement of PKA signaling in the second phase of insulin exocytosis. All these data provide some new insights into the mechanism by which current therapeutics may be improving insulin secretion.

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1. Introduction

In response to a sharp elevation of glucose, insulin secretion follows a biphasic time course [1,2]. An initial transient phase of secretion is completed within 10 min and is thought to be due to a "Readily Releasable Pool" (RRP) of docked and primed Large Dense Core Vesicles (LDCVs) at the plasma membrane [2]. This is followed by a slow developing and sustained second phase that involves a granule pool deeper within the cell, the "Reserve Pool" (RP) [2,3]. Thus, this second phase is presumed to require mobilization of RP granules to the cell surface to sustain insulin release for the duration of glucose stimulation. Thousands of LDCVs are located behind a filamentous sub-cortical actin barrier (F-actin) in the pancreatic β-cell, and F-actin remodeling is known to mobilize granules to the target membrane Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) sites at the cell surface [3], although the precise mechanisms involved in F-actin remodeling and granule mobilization has not been yet elucidated.

In type 2 diabetes (T2D), chronic hyperglycemia has been suggested to be detrimental to β-cell function leading to a reduced glucose-stimulated insulin secretion (GSIS) [4,5]. The deterioration of β-cell function as a result of glucotoxicity [6] is implicated in pathological progression of the disease [1,7]. In chronically hyperglycemic conditions, insufficient amounts of insulin secretion appear to be due both to reduction in the number of exocytotic events and to a premature termination of the fusion process [8]. Moreover, final steps in insulin exocytosis have been reported to be impeded since the expression of SNAREs proteins in pancreatic islets is found to be decreased in pancreatic β-cell lines [9], in

rat models of T2D [10–13] and in diabetic patients [14]. In these same studies, it is interesting to note that islet actin expression is also increased [11,14].

The incretin hormone glucagon-like peptide-1-(7–36) amide (GLP-1) and analogs have widely been used in the treatment of T2D [15,16]. However the mechanisms underlying the insulinotropic action of GLP-1 under glucotoxic conditions are little understood. Previous studies from our laboratory have shown that GLP-1, which potentiates GSIS via cAMP pathway, is able to restore the insulin-secretory glucose competence of the diabetic pancreatic ß-cells [17,18]. Recently, Kong et al. disclosed a novel mechanism whereby GLP-1 potentiates glucotoxicity-diminished GSIS by depolymerizing F-actin cytoskeleton in INS-1E ß-cell line [19].

Based on these observations, we now aim to focus on: 1/the impact of glucotoxicity on the F-actin and SNARE protein interactions; 2/the dynamics of glucose-induced insulin granule fusion and the F-actin web rearrangements, in INS-1 832/13 ß-cells exposed or not to glucotoxicity and 3/the potential rescue of exocytosis by GLP-1 and the involved pathways.

2. Materials and methods

2.1. Chemicals and plasmids

Culture products were from Lonza (Bâle, Switzerland). NPY-EGFP plasmid was a gift from Dr. S Barg (Uppsala University, Sweden). LifeAct-RFP plasmid was from Ibidi-Gmbh (Munich, Germany). Lipofectamine 2000 was purchased from Invitrogen (Saint Aubin, France). Jasplakinolide and Latrunculin B were purchased from Millipore. Collagenase, thapsigargin, Cytochalasin B, GLP-1, mouse monoclonal syntaxin 1 and actin antibodies were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Polyclonal syntaxin 1A antibody and monoclonal SNAP-25 antibody were purchased from Enzo Life Sciences (Villeurbanne, France). Polyclonal VAMP-2 and Cyclophilin A antibodies were from Synaptic System (Goettingen, Germany) and Millipore (Guyancourt, France) respectively. Protein G-plus agarose was obtained from Santa-Cruz (Heidelberg, Germany). Annexin-FITC and Propidium Iodide (PI) were purchased from BD Biosciences (Le Pont de Claix, France). ESI-05 (4-Methylphenyl-2, 4, 6-trimethylphenylsulfone) and Rp8 (8-Bromoadenosine-3′,5′-cyclic monophosphothioate, Rp isomer) were purchased from Biolog Life Science (Bremen, Germany).

2.2. INS-1 832/13 β-cell culture

The rat insulinoma ß-cell line, INS-1 832/13, was used between passages 20 and 40. As described [20], cells were cultured at 5% CO2–95% air at 37 °C in RPMI 1640 medium containing 11 mM D-glucose supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, 100 U/ml penicillin–streptomycin, 10 mM HEPES, 1 mM sodium pyruvate, 2 mM L-Glutamine and 50 μM beta-mercaptoethanol (Invitrogen, Saint Aubin, France). Glucotoxicity conditions were obtained by exposing INS-1 832/13 cells to 20 mM glucose (High Glucose; HG) during 96 h. Control cells were INS-1 832/13 cells exposed to standard culture medium with 11 mM glucose (Control; C) during 96 h. Each respective culture medium was changed after 48 h and replaced with the same culture medium until 96 h.

2.3. Rat islets isolation

All animal experimentation was conducted on fed, age-matched male diabetic Goto-Kakizaki (GK/Par) rats [21] and nondiabetic Wistar (W) rats maintained at the University Paris-Diderot animal core (Agreement A-75-13-17). The experimental protocol was approved by the institutional Animal Care and Use Ethical Committee of the Paris-Diderot University (registration number CEEA-40), in accordance with accepted standards of animal care as established in the French National Center for Scientific Research (CNRS) guidelines. Pancreas from rats (3-month-old) were digested with collagenase in Hank's balanced salt solution (HBSS, 137 mM NaCl, 5.36 mM KCl, 4.17 mM NaHCO3, 0.88 mM MgSO4, 0.44 mM KH2PO4, 0.34 mM Na2HPO4, 1.27 mM CaCl2, 10 mM Hepes, and 0.5% Bovine Serum Albumin) and islets of Langerhans purified from exocrine tissue by discontinuous density-gradient centrifugation (Histopaque 1077, Sigma-Aldrich, Saint-Quentin Fallavier, France) [22]. The islets were hand-picked under a stereomicroscope and frozen until used.

2.4. Measurements of insulin release by static incubations

INS-1 832/13 cells cultured in control (C) or HG conditions were washed in 1 ml KRBH buffer with 2.8 mM glucose and preincubated during 1 h in 1 ml of the same buffer (basal medium). Insulin secretion was then measured by using static incubations for indicated period, in 1 ml of KRBH-BSA containing 2.8 or 16.7 mM glucose, alone or with drugs as indicated in the figure legends. Fractions were then collected and stored at −20 °C. Insulin secretions and content were quantified using a radioimmunoassay kit (Millipore, Guyancourt, France). The amount of insulin secretion was normalized by cellular insulin content.

2.5. Assay of F-actin in vitro

F-actin content was measured by G/F-actin assay. After stimulation (conditions indicated in figures legends), INS-1 832/13 cells were homogenized in F-actin stabilization buffer equilibrated at 37 °C (50 mM PIPES, 50 mM NaCl, 5 mM MgCl2, 5 mM EGTA, 5% glycerol, 0.1% Nonidet P-40, 0.1% Triton X-100, 0.1% Tween 20, 0.1% β-mercaptoethanol, 1 mM ATP and protease inhibitor mixture) followed by centrifugation for 1 h at 100,000 × g at 37 °C to separate the F-actin from the G-actin pool. Supernatant was saved for analysis of the G-actin fraction, and the F-actin fraction was obtained from the cell pellet that was resuspended in denaturing F-actin solution (8 M urea, 15 μmM β-mercaptoethanol, 10 mM Tris pH 8) for 1 h at 4 °C. Equal amounts of both the supernatant (G-actin) and the resuspended pellet (F-actin) were subjected to western blotting with the use of an anti-actin antibody. The proportion of F-actin was quantified by scanning densitometry, using ImageJ 1.48v (National Institutes of Health, http://rsb.info.nih.gov/ij).

2.6. Co-immunoprecipitation experiments

Frozen islets or INS-1 832/13 cells from static incubations were harvested in Nonidet P-40 lysis buffer and lysates for subsequent use in co-immunoprecipitation experiments. Lysates were combined with mouse anti-Syntaxin 1 antibodies for 2 h at 4 °C followed by a second incubation with protein G Plus-agarose for 2 h. The resultant immunoprecipitates were subjected to 12% SDS-PAGE followed by transfer to PVDF membranes for immunoblotting, as described.

2.7. Western blotting

After 96 h of culture with the standard culture medium (11 mM glucose) or HG culture medium (20 mM glucose), INS-1 832/13 cells were lysed in Nonidet P-40 lysis buffer (100 mM NaCl, 50 mM Tris–HCl pH 7.4, 5 mM EDTA pH 8, 50 mM NaF, 1 mM NaOV, 1% Nonidet P-40 and 20% inhibitor cocktail) and lysates were cleared of insoluble material by microcentrifugation for 10 min at 4 °C. Equal amount of cleared lysate samples were homogenized in Leammli buffer and separated on 8–12% gradient SDS-PAGE, transferred to PVDF membrane and separated proteins identified by specific primary antibodies: β-actin (1/5000), syntaxin 1A (1/1000), SNAP-25 (1/1000), VAMP-2 (1/1000) and cyclophilin A (1/2000). Protein bands were then visualized by enhanced chemiluminescene (ECL plus, Pierce, Villebon-sur-Yvette, France).