Differential expression of E-cadherin at the surface of rat β-cells as a marker of functional heterogeneity

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Abstract

The aim of this study was to assess whether the expression of E-cadherin at the surface of rat β-cells is regulated by insulin secretagogues and correlates with insulin secretion. When cultured under standard conditions, virtually all β-cells expressed E-cadherin observed by immunofluorescence, but heterogeneous staining was observed. Using fluorescence-activated cell sorting (FACS), two β-cell sub-populations were sorted: one that was poorly labeled (‘ECad-low’) and another that was highly labeled (‘ECad-high’). After 1-h stimulation with 16.7 mM glucose, insulin secretion (reverse hemolytic plaque assay) from individual ECad-high β-cells was higher than that from ECad-low β-cells. Ca2+-dependent β-cell aggregation was increased at 16.7 mM glucose when compared with 2.8 mM glucose. E-cadherin at the surface of β-cells was increased after 18 h at 11.1 and 22.2 mM glucose when compared with 2.8 mM glucose, with the greatest increase at 22.2 mM glucose + 0.5 mM isobutylmethylxanthine (IBMX). While no labeling was detected on freshly trypsinized cells, the proportion of stained cells increased in a time-dependent manner during culture for 1, 3, and 24 h. This recovery was faster when cells were incubated at 16.7 vs 2.8 mM glucose. Cycloheximide inhibited expression of E-cadherin at 2.8 mM glucose, but not at 16.7 mM, while depolymerization of actin by either cytochasin B or latrunculin B increased surface E-cadherin at low glucose. In conclusion, these results show that expression of E-cadherin at the surface of islet β-cells is controlled by secretagogues including glucose, correlates with insulin secretion, and can serve as a surface marker of β-cell function.


Introduction

E-cadherin is a calcium-dependent transmembrane protein involved in homotypic cell–cell interactions (Angst et al. 2001). It is a member of the cadherin superfamily that is mainly expressed in the epithelial cells of many tissues, including endocrine pancreas (Rouiller et al. 1991). This adhesive molecule allows neighboring cells to stick together, but more interestingly, it is also involved in the regulation of signaling events (Ozawa & Kemler 1992, Aberle et al. 1996). The distal cytoplasmic domain of E-cadherin is bound to intracellular proteins known as catenins that serve to link the complex to the actin cytoskeleton (Ozawa & Kemler 1992, Aberle et al. 1996), to other proteins involved in signal transduction, and to nuclear transcription factors (Behrens et al. 1996). Thus, E-cadherin has been shown to play a central role in the development and maintenance of epithelial morphology, cell differentiation, migration proliferation, and apoptosis.

In the endocrine pancreas, adhesion molecules have been thought to be responsible for segregation during histogenesis of the different types of insulin cells (Rouiller et al. 1990). By interfering with endogenous cadherin activity in β-cells during pancreatic development, the initial clustering of β-cells and the normal localization of glucagon-producing α-cells in the periphery of pancreatic islets, which normally begins at E13.5–E14.5 days, were perturbed (Dahl et al. 1996, Esni et al. 1999). In addition, the loss of E-cadherin expression coincides with the transition from well-differentiated adenoma to invasive carcinoma in a transgenic mouse model of pancreatic β-cell carcinogenesis (Perl et al. 1998). Adhesion molecules are thought to be involved in β-cell activity as well. Rapid expression of polysialylated (PSA)-neural cell adhesion molecule (NCAM) at the surface of β-cells was observed under conditions that stimulate insulin secretion (Kiss et al. 1994) and differential expression of PSA-NCAM in the β-cell population correlates with differences in glucose responsiveness (Bernard-Kargar et al. 2001). In addition, blockade of E-cadherin-mediated cell adhesion in pancreatic islets abolishes glucose-stimulated increases in intracellular Ca2+ levels and insulin secretion, suggesting that loss of E-cadherin in β-cells is associated with impaired insulin secretion (Yamagata et al. 2002). Finally, expression levels of
E-cadherin were reduced in animal models of type 2 diabetes in which the islet architecture was perturbed (Shih et al. 2002).

This work was designed to study E-cadherin in β-cells from the adult rat, in order to assess whether the expression of this molecule at the surface of β-cells is regulated by glucose and correlates with insulin secretion.

**Materials and Methods**

**Islet isolation and β-cell purification**

Islets of Langerhans were isolated by collagenase digestion of pancreases from male Sprague–Dawley rats (weighing 180–200 g), followed by Ficoll purification using a modification of previously described procedures (Sutton et al. 1986, Rouiller et al. 1990). This protocol was approved by the Geneva veterinary authorities. For cell preparation, the isolated islets were rinsed thrice with Mg\(^{2+}\), Ca\(^{2+}\)-free PBS and resuspended in 1–5 ml of the same buffer containing 0.016% of trypsin (activity against casein, 1:250) and 0.0066% EDTA (Gibco, Life Technologies).

Digestion (with occasional pipetting) was for 6 min at 37°C and was stopped by the addition of 10 ml ice-cold Krebs–Ringer bicarbonate buffer, 10 mM Hepes, pH 7.4 (KRB), containing 0.5% BSA and 2.8 mM glucose. β-Cells were then separated from non-β-cells by auto-fluorescence–activated sorting using a FACStar-Plus cell sorter (Becton–Dickinson, San Jose, CA, USA), as previously described (van de Winkel & Pipeleers 1983, Rouiller et al. 1990), resulting in a population comprising 95–98% (insulin-positive) β-cells.

**Standard cell culture**

Sorted β-cells were washed twice in 10–15 ml sterile Dulbecco's minimum essential medium (DMEM; Gibco, Life Technologies), containing 11.1 mM glucose and 10% heat-inactivated fetal calf serum and supplemented with 110 U/ml penicillin, 110 µg/ml streptomycin, and 50 µg/ml gentamicin (complete DMEM). After centrifugation for 10 min at 130g, aliquots of 10⁵ cells were seeded in non-adherent 60 mm diameter Petri dishes containing 3 ml medium. Cells were then incubated for 18–24 h at 37 °C to allow full recovery of any cell surface molecules that may have been lost or damaged during islet isolation or cell purification.

**Immunofluorescence labeling on pancreas sections and isolated β-cells**

Samples of Sprague–Dawley rat pancreases were frozen in liquid nitrogen. Cryosections (5 mm thick) were then prepared, attached to slides, and fixed for 10 min at room temperature with a solution of 4% parafomaldehyde in PBS. Purified β-cells, attached in Cunningham's chambers, were rinsed with PBS and fixed or not with 4% paraformaldehyde for 20 min. Cryosections and fixed cells were then treated with 0.1% Triton X-100 in PBS for 10 min and preincubated for 30 min at room temperature in PBS containing 0.1% BSA. These preparations were exposed for 2 h at room temperature to a rabbit anti-E-cadherin polyclonal antibody prepared as previously described (Batz & Kemler 1994) and diluted 1:200 in PBS containing 0.1% BSA. After three washes in PBS, they were incubated for 1 h at room temperature with a fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibody (Sigma), diluted 1:100 in PBS containing 0.1% BSA and finally washed thrice with PBS. For living (non-fixed) cells attached to Cunningham's chambers treatment with Triton X-100 and preincubation were omitted, and both incubations with primary and secondary antibodies were performed at 4°C for 1 h. Cells were then fixed 20 min with 4% paraformaldehyde and rinsed thrice with PBS.

The specificity of the anti–E-cadherin serum has been tested i) on protein extract of insulin–expressing cells analyzed by western blotting, it recognizes a major band with apparent molecular weight of about 120 kDa, corresponding to the MW of E-cadherin (not shown); ii) on pancreas section (see Fig. 1), only epithelial tissue was labeled and staining was restricted to the cell membrane; and iii) pre-immune serum from the same animal did not stain any cell or tissue.

In some cases, parafomaldehyde-fixed cells were submitted to an immunofluorescence for insulin. To this end, cells were permeabilized with Triton X-100 (0.1%, 10 min) and then incubated for 2 h with an anti-insulin guinea pig serum prepared as previously described (Wright et al. 1968), (1:600), and 1 h with a rhodamine-conjugated goat anti-guinea pig antibody (1:400). All preparations were examined by fluorescence microscopy (Zeiss Axiophot, Oberkochen, Germany).

To assess whether insulin and E-cadherin co-localized into the same granules, parafomaldehyde–fixed cells were first permeabilized with Triton X-100 (0.1%, 10 min), and then exposed to the same anti-insulin and anti-E-cadherin antibodies and secondary antibodies described above. Preparations were examined by confocal microscopy (LSM510 Meta, Zeiss).

**E-cadherin expression tested by FACS**

Sorted β-cells were washed twice with complete DMEM and incubated for 18 h at 37°C in complete DMEM containing either 2.8 mM, 11.1 mM, 22.2 mM glucose, or 22.2 mM glucose and 0.5 mM isobutylmethylxanthine (IBMX); the cells were incubated at low density (10⁵ cells/10 ml) in non-adherent 10 cm diameter Petri dishes in order to prevent aggregation. The cells were then rinsed with cold KRB medium supplemented with 0.1% BSA and 2.8 mM glucose (KRB) and incubated for 45 min at 4°C with the rabbit anti-E-cadherin antibody or a non-immune rabbit serum, diluted 1:200 in KRB. The cells were then rinsed thrice with KRB and incubated again for 45 min at 4°C with a FITC-conjugated-goat anti-rabbit antibody (Sigma), diluted 1:100 in KRB. Then, the cells were rinsed thrice with KRB and analyzed using a FACSCalibur flow cytometer (Beckton–Dickinson).

**Trypsin treatment and E-cadherin expression recovery test**

β-Cells purified by fluorescence-activated cell sorting (FACS) and incubated 18–24 h under standard conditions were rinsed...
twice with PBS without Ca$^{2+}$ and Mg$^{2+}$ and incubated at 37°C for 6 min with the same buffer containing 0.016% of trypsin (activity against casein, 1:250) and 0.3 mM EDTA (Gibco, Life Technologies). The cells were rinsed with complete DMEM and resuspended in the same medium containing either 2.8 or 16.7 mM glucose and supplemented or not with 10 μM cycloheximide, 5 μg/ml cytochalasin B (Sigma), or 10 μg/ml latrunculin B (Calbiochem, Darmstadt, Germany). Then, the cells (10–15×10$^3$/50 μl) were introduced into Cunningham’s chambers, previously coated with poly-L-lysine and incubated for 1 or 3 h at 37°C. E-cadherin expression was then evaluated on living cells as described below.

**Aggregation assay**

After purification, β-cells were incubated for 18 h in complete DMEM containing 2.8 mM glucose; the cell density was too low (10$^3$ cells/10 ml in non-adherent 10 cm diameter Petri dishes) to prevent cell aggregation. The cells were then incubated for 45 min at 37°C in KRB buffer supplemented with 0.1% BSA containing either 2.8 or 16.7 mM glucose. The cells were washed either with control KRB or with Ca$^{2+}$-, Mg$^{2+}$-free KRB, supplemented with 0.1% BSA and 2.8 or 16.7 mM glucose. Then, the cells were submitted to an aggregation test as previously described (Rouiller et al. 1990). Briefly, cells were suspended in 150 μl of the same buffers at a concentration of 5×10$^5$/ml in 10 ml polycarbonate conical tubes (Nunc, Roskilden, Denmark). The tubes were then placed at a fixed angle (30°) in a shaking water bath (80 cycles/min) at 37°C for 60 min. Aggregation was assessed quantitatively by comparing the number of events before and after the aggregation period as measured in a ZM Coulter counter (Coulter Electronics, Luton, UK). The percentage of aggregation was given by the following formula: \((b-a)/b \times 100\), where \(b\) is the number of events before aggregation and \(a\) is the number of events counted after the 60-min aggregation period.
Fluorescence-activated cell sorting of β-cells with differential expression of E-cadherin on their surface

After their purification by FACS, β-cells were incubated for 24 h in complete DMEM, cells were cultured at low density (10^5 cells/10 ml in 10 cm diameter Petri dishes) to prevent their reaggregation. Cells were then rinsed with PBS supplemented with 0.1% BSA and incubated for 60 min at 4 °C with the rabbit anti-E-cadherin antibody, diluted 1:200 in PBS–0.1% BSA. After three washes with PBS–0.1% BSA, cells were incubated again for 60 min at 4 °C with a FITC-conjugated goat anti-rabbit antibody (Sigma), diluted 1:160 in PBS–0.1% BSA. Finally, cells were rinsed thrice with PBS–0.1% BSA and sorted using a FACSStar-plus (Becton–Dickinson). An argon laser illuminated the cells at 488 nm and an interference filter detected the emission light at 510–540 nm. Forward light scatter and fluorescence channel 1 were used to analyze the cells in dot plots. Two windows were set to enclose the high-labeled and the low-labeled β-cells respectively. Sorted cells were then rinsed twice with complete DMEM and incubated in the same medium for 24 h before processing for reverse hemolytic plaque assay (RHPA). In one experiment, proteins were extracted with acid–ethanol from 80,000 cells from each cell population and cellular insulin content was measured by ELISA.

Insulin secretion by RHPA

Insulin secretion of β-cells was assessed by a RHPA, as previously described (Salomon & Meda 1986, Bosco & Meda 1998). Briefly, β-cells were diluted in KRB buffer supplemented with 0.1% BSA and 2.8 mM glucose. Five percent (v/v) of packed sheep red blood cells (Behring Institute, Marburg, Germany) previously coated with protein A were then mixed with β-cells, and 50–60 µl of this preparation were introduced into Cunningham’s chambers. After 1-h incubation at 37 °C, the chambers were first rinsed with KRB containing either 2.8 or 16.7 mM glucose, and then filled with the same buffer, supplemented with a heat-inactivated (45 min at 56 °C) anti-insulin guinea pig serum (Wright et al. 1968), (1:50). After 1-h incubation at 37 °C, chambers were rinsed with KRB containing 2.8 mM glucose, filled with the same buffer containing guinea pig complement (1:40, Behring Institute), and incubated at 37 °C for 1 h. Chambers were then filled with a 0–0.04% (w/v) solution of trypan blue in KRB, rinsed with KRB, and filled with 4% paraformaldehyde. Analysis was restricted to single cells that excluded trypan blue at the end of the plaque assay. Results are expressed as total plaque development, which represents the total plaque area formed by 100 cells (Bosco & Meda 1998).

Statistical analysis

Data are presented as mean ± S.E.M. for n independent experiments, and levels of significance for differences between groups were assessed by Student’s t-test for unpaired groups. Where appropriate, differences were tested by ANOVA followed by a post hoc Scheffe test.

Results

Heterogeneous expression of E-cadherin at the surface of β-cells

When immunostaining of E-cadherin was performed on pancreas sections, both exocrine tissue and islets were shown to be labeled (Fig. 1A). In both instances, labeling was confined to the cell membrane and mainly localized to cell-to-cell contacts. Within islets, expression of E-cadherin was rather heterogeneous, with some cells showing a strong and others a weak labeling. In many islets (19/30 from four different pancreases), the strongest labeling was on the more centrally located cells (Fig. 1A, E and F). To further assess the expression of E-cadherin in β-cells, immunostaining was performed on isolated β-cells purified by FACS. In this case too, heterogeneity was obvious (Fig. 1B and C). Single and aggregated β-cells displayed a surface staining (Fig. 1B and C) that became clearly stronger at the cell-to-cell contacts when labeling was performed on permeabilized cells (Fig. 1D). By double staining for E-cadherin and insulin, it was confirmed that low and high E-cadherin expressing cells were β-cells (Fig. 2A–C). On permeabilized cells, we observed a granular cytoplasmic labeling for E-cadherin by conventional light microscopy that could have been accounted for by background labeling. However, by confocal microscopy, it was apparent that some of these E-cadherin dots were also labeled for insulin (Fig. 2D and E). This suggests that E-cadherin and insulin can be co-localized within the same granules.

Insulin secretion from β-cell subpopulations sorted according to their E-cadherin expression

Due to the above-described heterogeneity of E-cadherin expression and to the well-known heterogeneity of insulin secretion from isolated β-cells (Salomon & Meda 1986, Bosco et al. 1989), we wondered whether there was a correlation between E-cadherin expression and insulin secretion. To address this, β-cells were sorted according to their E-cadherin expression as illustrated in Fig. 3A. Thus, two cell subpopulations were obtained expressing low (′ECAd-low′) and high (′ECAd-high′) levels of E-cadherin. Then, the two subpopulations were tested for their ability to secrete insulin using a RHPA. At low glucose concentration (2.8 mM), the total plaque development (which represents the total plaque area formed by 100 β-cells) was low (~10 × 10^3 µm^2) for both subpopulations. After 1-h stimulation with 16.7 mM glucose, the total plaque development was about 100 × 10^3 µm^2 for the ECAd-high cells and significantly lower (about 50 × 10^3 µm^2) for the ECAd-low cells (Fig. 3B). Cellular insulin content measured by ELISA on extracts from 80,000 cells in a single control experiment, was 4.5 and 3.25 pg/cell for ECAd-low and ECAd-high cells respectively and as such if anything
higher in the ECad-low population that secreted less insulin in response to glucose. This indicates that there is a correlation between the expression of E-cadherin at the surface of b-cells and glucose-induced insulin secretion that is not related to a corresponding change in insulin content.

**Effect of glucose on E-cadherin expression at the surface of b-cells**

This set of experiments was aimed to assess whether insulin secretagogues modulated E-cadherin expressed at the surface of b-cells. Isolated b-cells were incubated for 18 h at either low (2.8 mM), intermediate (11.1 mM), or high (22.2 mM) glucose concentrations; the last condition was supplemented or not with 0.5 mM IBMX. Then, cell surface expression of E-cadherin was analyzed by FACS after immunofluorescence performed on living b-cells. Figure 4A shows the distribution of E-cadherin fluorescence in the b-cell population incubated under these different conditions. With increasing glucose concentrations, the distribution of E-cadherin staining showed a shift toward higher fluorescence values. The presence of IBMX induced the highest shift. Quantitative results (Fig. 4B) show that the mean fluorescence value was increased by 50% for both 11.1 and 22.2 vs 2.8 mM glucose and by 100% for 22.2 mM glucose supplemented with IBMX versus 2.8 mM glucose. These results show that insulin secretagogues (glucose and IBMX) have a positive effect on the expression of E-cadherin.

To understand better the effect of glucose on E-cadherin expression, isolated b-cells were treated with trypsin to remove membranous E-cadherin. Then, E-cadherin recovery at the cell surface was tested by immunofluorescence and microscopic analysis. Immediately after trypsin treatment, virtually no cells expressed E-cadherin (not shown). With time, the percentage of labeled cells gradually increased, and virtually all cells were found labeled after 18 h of culture in the presence of 2.8 or 16.7 mM glucose (Fig. 5). After 1 and 3 h of incubation, the percentage of labeled cells was higher in the presence of 16.7 mM glucose when compared with 2.8 mM glucose. These data indicate that re-expression of E-cadherin at the surface of b-cells occurs at both low and high glucose concentrations, but is accelerated under conditions that stimulate insulin secretion (16.7 mM glucose).
Effect of glucose on β-cell aggregation

To show that E-cadherin expressed at the β-cell membrane was functionally active, an aggregation test was performed with β-cells pre-incubated for 45 min at either 2.8 or 16.7 mM glucose. When the test was run in the presence of Ca²⁺, aggregation of cells pre-incubated at 16.7 mM glucose was significantly higher than that of cells pre-incubated at 2.8 mM glucose. In absence of Ca²⁺, aggregation was significantly lower and no difference was observed between 2.8 and 16.7 mM glucose, indicating that only cell aggregation mediated by Ca²⁺-dependent adhesion molecules was affected by glucose.

Effect of cycloheximide on the expression of E-cadherin

The time-dependent appearance of E-cadherin at the β-cell surface under non-stimulated conditions suggests that its expression is, at least in part, constitutive. To further verify this hypothesis and to determine whether this depended upon synthesis of new proteins, the effect of the protein synthesis inhibitor cycloheximide was tested on the recovery of E-cadherin expression at the cell surface under basal conditions. However, at 16.7 mM glucose, cycloheximide did not inhibit recovery of E-cadherin expression to the same extent as in the presence of 2.8 mM glucose.
E-cadherin (Fig. 7). This suggests that a pool of intracellular E-cadherin may be present in β-cells and could be expressed at the cell membrane only after glucose stimulation.

**Effect of cytochalasin and latrunculin on the expression of E-cadherin**

Depolymerization of actin filaments lying beneath the β-cell membrane has been shown to promote exocytosis of insulin granules (Malaisse & Orci 1979, Howell & Tyhurst 1986). In order to assess whether depolymerization of this actin network could affect E-cadherin expression, β-cells were treated with trypsin, and recovery of E-cadherin at the cell membrane was studied in the absence or presence of drugs that depolymerize actin filaments: cytochalasin-B and latrunculin B. When trypsinized, and successively incubated for 3 h at 2.8 mM glucose, 20% of β-cells expressed E-cadherin. Under this glucose concentration, the percentage significantly increased up to 40–50% in the presence of cytochalasin or latrunculin (Fig. 8). After 3 h at 16.7 mM glucose, the percentage of β-cells expressing E-cadherin was

Figure 5 Effect of glucose on the expression of E-cadherin at the surface of β-cells after trypsin treatment. β-Cells were treated with trypsin and incubated for 1, 3, or 18 h in medium containing 2.8 (white columns) or 16.7 mM glucose (black columns). The cells were then labeled by immunofluorescence for E-cadherin and the percentage of labeled cells were determined. Data are mean ± S.E.M. of five, six, and three independent experiments for 1, 3, and 18 h respectively. At both 2.8 and 16.7 mM glucose, the percentage of labeled cells significantly increased with time in culture (*P<0.02–0.0001). At 1 and 3 h, the percentage of labeled cells was higher at 16.7 vs 2.8 mM glucose (**P<0.02–0.005).

Figure 6 Effect of glucose on β-cell aggregation. β-Cells were incubated in static conditions for 18 h in culture medium containing 2.8 or 16.7 mM glucose; the cells were then transferred in KRB containing either 2.8 or 16.7 mM glucose, incubated for 45 min at 37 °C, and submitted to the aggregation test under the same glucose concentration and in the presence or absence of Ca²⁺. Data are mean ± S.E.M. of four independent experiments; in the presence of Ca²⁺, aggregation was higher (*P<0.002) in 16.7 vs 2.8 mM glucose; in the absence of Ca²⁺, aggregation was significantly diminished (**P<0.005) and no effect of glucose was observed.

Figure 7 Effect of cycloheximide on the expression of E-cadherin at the surface of β-cells. β-Cells were treated with trypsin and incubated for 1 and 3 h in culture medium containing 2.8 or 16.7 mM glucose, supplemented (black columns) or not (white columns) with 10 μM cycloheximide. The cells were then labeled by immunofluorescence for E-cadherin and the percentage of labeled cells was determined. Data are mean ± S.E.M. of three independent experiments. At 2.8 mM glucose, cycloheximide significantly decreased the percentage of labeled cells (***P<0.04 for 1 h, and **P<0.005 for 3 h).

Figure 8 Effect of cytochalasin B and latrunculin on the expression of E-cadherin at the surface of β-cells. β-Cells were treated with trypsin and incubated for 3 h in medium containing 2.8 or 16.7 mM glucose and supplemented or not with 5 μg/ml cytochalasin B or 10 μM latrunculin B. The cells were then labeled by immunofluorescence for E-cadherin and the percentage of labeled cells were determined. Columns are means ± S.E.M. of three independent experiments. At 2.8 mM glucose, cytochalasin B (*P<0.03) and latrunculin (**P<0.01) significantly increased the percentage of labeled cells versus control condition.
already elevated in the absence of cytochalasin or latrunculin and was not further increased in the presence of these drugs (Fig. 8). These data show that even in absence of metabolic activation by glucose, it is possible to improve the expression of E-cadherin at the cell surface with drugs that depolymerize cortical actin and thereby favoring access of granules to the membrane for exocytosis.

Discussion

In this work, we show that the expression of E-cadherin at the surface of β-cells is heterogeneous, regulated by secretagogues including glucose and correlates with insulin secretory capacity from individual β-cells. We base these conclusions on several lines of evidence. Heterogeneity of surface labeling of β-cells with anti-E-Cadherin was evident in vitro on dispersed cells but also in situ on pancreas sections, suggesting that this is not an artefact of islet isolation or more specifically of the procedure for sorting β-cells by FACs. When E-cadherin was stripped from β-cells by trypsinization, the rate of reappearance of this adhesion molecule at the cell surface was quite variable, suggesting that either the rate of E-cadherin synthesis or of its recycling differs between β-cells. We attempted to distinguish between these alternatives by inhibiting protein synthesis with cycloheximide. The results of such inhibition indicated that the reappearance of E-cadherin at low glucose is entirely dependent on protein synthesis, whereas at high glucose, this dependence is only partial. It is concluded that glucose stimulates recycling of E-cadherin from an intracellular pool of proteins that is involved in insulin granule exocytosis and is already elevated in the absence of cytochalasin or latrunculin. These results are in accord with those of Aberle et al. (1996) that showed a correlation between the abundance of PSA-Ncam and insulin secretion. When we measured insulin secretion by RHPA, only single β-cells were analyzed. Under these circumstances, there would be no contribution of E-cadherin signaling toward β-cell function, since this adhesion molecule is active only when it is engaged in Ca2+-dependent homophilic engagement of another molecule on a neighboring cell. We thus believe that cells with high levels of E-cadherin do so as a result of their high secretory capacity and not the contrary. In a previous work, using similar FACs and RHPA technologies used in this study, we demonstrated that insulin secretory activity of single β-cells correlated with their size (Giordano et al. 1993). Here, a correlation between β-cell size and E-cadherin expression cannot be excluded. FACs analysis of dissociated and E-cadherin-labeled β-cells (Fig. 3A) may suggest that highly labeled β-cells were bigger than poorly labeled β-cells. Further analyses by microscopy and morphometry are needed to confirm this.

Cell-to-cell adhesion is known to improve insulin secretion (Bosco et al. 1989). Here, we show that E-cadherin at the cell surface is functionally active. Indeed, β-cells were able to reaggregate in a calcium-dependent manner and glucose increased aggregation in a dose-dependent fashion. Our hypothesis is that glucose activates E-cadherin expression at the surface of β-cells, leading to the development of cell-to-cell contacts and/or reinforcement of signaling via E-cadherin. In turn, these events could improve glucose-induced insulin secretion. Our results further identify E-cadherin as a new surrogate cell surface marker for β-cell function that may be useful in the development of noninvasive imaging technology for clinical assessment of functional β-cell mass.

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