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. Ca^{2+} -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 cytochalasin 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.

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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 insular 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 Ca^{2+} 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 autofluorescence-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 μ g/ml streptomycin, and 50 μ g/ml gentamicin (complete DMEM). After centrifugation for 10 min at 130 *g*, aliquots of 10^5 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% paraformaldehyde 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 (Butz & 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, paraformaldehyde-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, paraformaldehyde-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 (10^5 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 (Becton–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

Figure 1 Heterogeneous expression of E-cadherin at the surface of β -cells. (A) Sections of rat pancreas showing labeling for E-cadherin at the cell surface in both exocrine and endocrine tissues. The islet of Langerhans, delimited by arrowheads, presents cells that are heterogeneously labeled. (B and C), Living purified β -cells labeled for E-cadherin. Coexistence of weak and strong labeling is evident in both aggregated (B) and single β -cells (C). (D), Immunostaining on a fixed and permeabilized aggregate of two β -cells showing a strong labeling at the cell–cell contact. (E and F), Section of rat pancreas co-stained for E-cadherin (green in E) and insulin (red in F); a specific cell surface labeling for E-cadherin was present in islet (i) and exocrine tissue, including pancreatic duct (d), and was absent in artery (a) and vein (v). The bar represents 40 μ m in A, 15 μ m in B, C and D, and 80 μ m in E and F.

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 μ M cycloheximide, 5 μ g/ml cytochalasin B (Sigma), or 10 μ g/ml latrunculin B (Calbiochem, Darmstadt, Germany). Then, the cells ($10\text{--}15 \times 10^3/50 \mu\text{l}$) 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 (10^5 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 μ l of the same buffers at a concentration of $5 \times 10^5/\text{ml}$ 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 FACStar-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 μ l 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 0.04% (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 $\pm$ 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* Scheffé 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 ($\sim 10 \times 10^3 \mu\text{m}^2$) for both subpopulations. After 1-h stimulation with 16.7 mM glucose, the total plaque development was about $100 \times 10^3 \mu\text{m}^2$ for the ECad-high cells and significantly lower (about $50 \times 10^3 \mu\text{m}^2$) 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

Figure 2 Colocalization of E-cadherin and insulin. (A–C) Fluorescence-microscopy views of a β -cell pair comprising one cell with a stronger membrane labeling for E-Cadherin than the other. (A) Living cells were first labeled for E-cadherin using a FITC-conjugated secondary antibody (green on original figure). (B) Then, cells were fixed, permeabilized, and labeled for insulin using a rhodamine-conjugated secondary antibody (red on original figure). (C) Merged figures. (D, E) Confocal microscopy views of a β -cell pair after fixation, permeabilization, and labeling for E-cadherin (D) and insulin (E). E-cadherin was labeled using a FITC-conjugated secondary antibody (D, green on original figure), and insulin was labeled using a rhodamine-conjugated secondary antibody (E, red on original figure). Merged figures are shown in (F). Arrows show granules that are labeled for both E-cadherin and insulin and appearing in yellow on original merged figure.

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 β -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 β -cells

This set of experiments was aimed to assess whether insulin secretagogues modulated E-cadherin expressed at the surface of β -cells. Isolated β -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 β -cells. Figure 4A shows the distribution of E-cadherin fluorescence in the β -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 β -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 β -cells occurs at both low and high glucose concentrations, but is accelerated under conditions that stimulate insulin secretion (16.7 mM glucose).

Figure 3 Insulin secretion from β -cell subpopulations sorted according to their E-cadherin expression. (A) Dot plot analysis of dissociated β -cells examined for their fluorescence and cell size. Left panel represents β -cells before and right panel β -cells after immunofluorescence labeling with an E-cadherin antibody. β -Cells presenting high and low fluorescence were sorted using the top and bottom windows respectively. (B) Insulin release from sorted β -cells with high and low E-cadherin labeling was studied using a RHPA after 1-h incubation at 16.7 mM glucose and expressed as total plaque development. Data are means $\pm$ S.E.M. of four independent experiments and show that β -cells expressing high levels of E-cadherin secreted twofold more insulin than β -cell expressing low levels (* $P < 0.02$).

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 (Fig. 6) at either 2.8 or 16.7 mM glucose. When the test was run in the presence of Ca^{2+} , 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 Ca^{2+} , aggregation was significantly lower and no difference was observed between 2.8 and 16.7 mM glucose, indicating that only cell aggregation mediated by Ca^{2+} -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

Figure 4 Effect of glucose on expression of E-cadherin at the surface of β -cells evaluated by FACS. (A) Distribution of E-cadherin fluorescence measured by flow cytometry on β -cells incubated at different glucose concentrations. Distribution 1 represents cells analyzed before immunostaining. Distributions 2–5 represent cells analyzed after immunostaining and previously incubated in the presence of 2.8 (distribution 2), 11.1 (distribution 3), 22.2 mM glucose (distribution 4), and 22.2 mM glucose supplemented with IBMX (distribution 5) for 18 h (B). Quantitative results of the experiments illustrated in (A). For each distribution, the mean fluorescence was calculated and expressed as the percent of the value obtained for 2.8 mM glucose. Data are mean $\pm$ S.E.M. of four independent experiments. Differences were tested statistically using ANOVA and *post hoc* Scheffe tests. Fluorescence is significantly higher at 11.1 (* $P < 0.04$) and 22.2 (** $P < 0.02$) vs 2.8 mM glucose and at 22.2 mM glucose supplemented with IBMX (*** $P < 0.005$) vs 2.8, 11.1, or 22.2 mM glucose alone.

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 after trypsin treatment of β -cells. When incubated at 2.8 mM glucose after trypsin treatment, cycloheximide completely inhibited the time-dependent rescue of E-cadherin expression (Fig. 7). These results argue in favor of a requirement for synthesis of new molecules of E-cadherin for its re-expression at the β -cell surface under basal conditions. However, at 16.7 mM glucose, cycloheximide did not inhibit recovery of

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 $\pm$ 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 (** $P < 0.02$ –0.01).

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

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 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 Ca^{2+} . Data are mean $\pm$ S.E.M. of four independent experiments; in the presence of Ca^{2+} , aggregation was higher (* $P < 0.002$) in 16.7 vs 2.8 mM glucose; in the absence of Ca^{2+} , 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 were determined. Data are mean $\pm$ 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).

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 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 $\mu\text{g}/\text{ml}$ 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 $\pm$ 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 previously existing protein, whereas reappearance of E-cadherin at low glucose is constitutive and dependent upon protein synthesis. Additional data are compatible with the pool recruited to the cell surface by high glucose being present on membranes of insulin secretory granules. Stimulation of insulin secretion, whether by glucose alone or the combination of high glucose and elevation of intracellular cAMP (IBMX) thus resulted in a graded increase in surface E-cadherin that correlates with the well-established stimulation of secretion by glucose with or without IBMX. A similar phenomenon has been demonstrated for another cell adhesion molecule, PSA-NCAM, that is presented at the surface of β -cells in response to secretagogues as a result of its localization to insulin granule membranes (Kiss *et al.* 1994).

It has been shown previously that β -cells are heterogeneous both in terms of deployment of surface proteins and insulin secretion. The amounts of both NCAM and integrins have thus been shown to differ from one β -cell to the next (Bosco *et al.* 2000, Bernard-Kargar *et al.* 2001), and functional heterogeneity has been documented by several groups (Salomon & Meda 1986, Stefan *et al.* 1987, Pralong *et al.* 1990, Soria *et al.* 1991, van Schravendijk *et al.* 1992). In the present study, the strongest labeling of E-cadherin was observed in the more central part of islets, corresponding to β -cells that have the higher insulin secretion activity (Stefan

et al. 1987). To further investigate whether a correlation does exist between E-cadherin expression and insulin secretion, we sorted by FACS a poorly labeled and a highly labeled β -cell population. By RHPA, we found that the highly E-cadherin expressing β -cells secreted more insulin than the other cells. These results are in accord with those of Bernard-Kargar *et al.* (2001) that showed a correlation between the abundance of PSA-NCAM expressed at the cell membrane 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 cell adhesion molecule is active only when it is engaged in Ca^{2+} -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 non-invasive imaging technology for clinical assessment of functional β -cell mass.

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