RESEARCH

Intercellular contacts affect secretion and biosynthesis of pancreatic islet cells

David Cottet-Dumoulin[1,2], **Quentin Perrier**[1,2], **Vanessa Lavallard**[1,2], **David Matthey-Doret**[1,2], **Laura Mar Fonseca**[1,2], **Juliette Bignard**[1,2], **Reine Hanna**[1,2], **Géraldine Parnaud**[1,2], **Fanny Lebreton**[1,2], **Kevin Bellofatto**[1,2], **Ekaterine Berishvili**[1,2], **Thierry Berney**[1,2] and **Domenico Bosco**[1,2]

[1]Cell Isolation and Transplantation Center, Department of Surgery, Geneva University Hospitals and University of Geneva, Geneva, Switzerland
[2]Diabetes Center of the Faculty of Medicine, University of Geneva, Geneva, Switzerland

Correspondence should be addressed to D Bosco: domenico.bosco@unige.ch

Abstract

Cell protein biosynthesis is regulated by different factors, but implication of intercellular contacts on alpha and beta cell protein biosyntheses activity has not been yet investigated. Islet cell biosynthetic activity is essential in regulating not only the hormonal reserve within cells but also in renewing all the proteins involved in the control of secretion. Here we aimed to assess whether intercellular interactions affected similarly secretion and protein biosynthesis of rat alpha and beta cells. Insulin and glucagon secretion were analyzed by ELISA or reverse hemolytic plaque assay, and protein biosynthesis evaluated at single cell level using bioorthogonal noncanonical amino acid tagging. Regarding beta cells, we showed a positive correlation between insulin secretion and protein biosynthesis. We also observed that homologous contacts increased both activities at low or moderate glucose concentrations. By contrast, at high glucose concentration, homologous contacts increased insulin secretion and not protein biosynthesis. In addition, heterogeneous contacts between beta and alpha cells had no impact on insulin secretion and protein biosynthesis. Regarding alpha cells, we showed that when they were in contact with beta cells, they increased their glucagon secretion in response to a drop of glucose concentration, but, on the other hand, they decreased their protein biosynthesis under any glucose concentrations. Altogether, these results emphasize the role of intercellular contacts on the function of islet cells, showing that intercellular contacts increased protein biosynthesis in beta cells, except at high glucose, and decreased protein biosynthesis in alpha cells even when glucagon secretion is stimulated.

Key Words

- protein synthesis
- pancreatic islet cell
- insulin secretion
- glucagon secretion
- azidohomoalanine

Journal of Endocrinology (2023) **258**, **e220304**

Introduction

Islets of Langerhans are the regions of the pancreas that contain endocrine cells. They are composed of four major cell types: alpha, beta, delta and pancreatic polypeptide (PP) cells, which secrete glucagon, insulin, somatostatin and PP, respectively. Insulin and glucagon are the main hormones involved in the regulation of blood glucose levels. Insulin decreases glucose levels in the bloodstream by inducing glucose storage in the liver, muscle and adipose tissue, while glucagon increases blood sugar levels by triggering glycogenolysis and neoglucogenesis mainly in the liver. The rapidly changing metabolic needs of the body are mainly met by a tight regulation of secretion from islet cells. The mechanisms involved in insulin and glucagon secretion have been extensively studied as a deficit or a surplus of these hormones is associated with diabetes. Secretion of these hormones is

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affected by intrinsic cellular glucose sensing, along with autonomic nervous inputs and other hormones from the gastrointestinal system. More recently, many studies have emphasized the role of intercellular interactions, such as paracrine and direct cell-to-cell (juxtacrine) contacts between islet cells on the secretion of insulin and glucagon (Bosco *et al.* 1989, Wojtusciszyn *et al.* 2008, Koh *et al.* 2012). In rodent islets, beta cells are located in the core, while alpha and delta cells form a mantle at the periphery. In human islets, alpha cells are more widespread throughout the islet. Despite these differences, the islet architecture of both species promote intercellular interactions that are crucial for adequate hormone secretion (Kim *et al.* 2009, Bosco *et al.* 2010, Lavallard *et al.* 2016). Many studies have revealed the positive effect of islet intercellular contacts on insulin secretion in rats, mice and humans (Bosco *et al.* 1989, Hauge-Evans *et al.* 1999, Wojtusciszyn *et al.* 2008). The availability of islet hormones is also controlled at the level of protein biosynthesis to satisfy the changing metabolic demand. The biosynthetic activity is essential in regulating not only the hormonal reserve within cells but also in renewing all the proteins involved in the control of secretion. In this regard, glucose has been shown to stimulate beta cell biosynthetic activity, including insulin synthesis. It has also been reported that insulin expression and biosynthesis are controlled by incretin hormones (Fehmann & Habener 1992). Additionally, previous studies have demonstrated that glucose directly triggers beta cell protein biosynthesis, independent of insulin secretion (Permutt & Kipnis 1972, Cottet-Dumoulin *et al.* 2021). However, the effect of cell interactions on islet cell biosynthetic activity remains largely unexplored. In this study, we aimed to assess the protein biosynthesis of rat islet cells using the BONCAT strategy that features the incorporation of azidohomoalanine (AHA) in nascent peptides. This method allows the quantification of biosynthetic activity in every individual cell, whilst permitting us to study the changes of protein biosynthesis in single cells and those in contact with other islet cell types.

Material and methods

Islet cell isolation and culture

Animal studies were approved by the Geneva Institutional Animal Care and Use Committee. Islets were isolated from male Sprague Dawley rats (350 g). Ten mg of collagenase (Sigma Aldrich) were resuspended in 10 mL of Hanks' balanced salt solution (Bichsel, Interlaken, Switzerland) and were injected into the pancreas via the common bile duct of euthanized rats. The pancreas was excised and digested 10 min at 37°C. Islets were purified by Ficoll density gradient separation and dissociated into cells by an incubation of 4 min at 37°C in a solution of 0.05% trypsin supplemented with 0.48 mM ethylenediaminetetraacetic acid (Gibco). Then, rat islet cells were either sorted by fluorescence-activated cell sorting (FACS) or incubated at a concentration of 100,000 cells/10 mL in 10-cm-non-adherent petri dishes for 24 h at 37°C with 5% CO_2 using Dulbecco's modified Eagle medium (DMEM) (Gibco) supplemented with 1% L-glutamine-penicillin-streptomycin solution (Gibco), 10% fetal bovine serum (FBS) (Gibco), 1% sodium pyruvate (Gibco) and 11.2 mM glucose (without other specification, hereafter this medium is referred as complete DMEM). This 24 h incubation period allowed us to obtain a cell population comprising single islet cells and aggregated islet cells into small clusters of two to ten cells.

Sorting of islet cells

Cells were sorted by FACS on the automated S3e™ Cell sorter (Bio-Rad). The first selection of cells was made on their light scattering properties (forward scatter and side scatter). Then, distinction between beta cells and non-beta cells was made on their auto-fluorescence difference for an emission between 515 and 545 nm following their excitation by the laser of 488 nm (Van de Winkel *et al.* 1982). When purity was tested by immunofluorescence using specific anti-insulin and anti-glucagon antibodies, beta cell population comprised about 99.9% beta cells and 0.1% non-beta cells while non-beta cell population comprised about 5% beta cells, 45% alpha cells, and unidentified cells. Finally, alpha cell population comprised about 96% alpha cells. In order to purify alpha cells from non-beta cells, a second selection was made on their light scattering in side scatter, as alpha cells emit a stronger signal in side scatter (greater granularity). Beta cells, non-beta cells and alpha cells were then directly used for pseudo-islet formation. Cells sorted by FACS were only used for pseudo-islet formation. When using reverse hemolytic plaque assay (RHPA) or AHA-labeing technics, each cell type was identified by immunostaining.

Pseudo-islet formation

After sorting, cells were rinsed with complete DMEM, centrifuged three times and then counted. Different combinations of sorted cells were placed into 256-micro-well agar plates with complete DMEM: 51,200 beta cells,

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25,600 non-beta cells or alpha cells, and 51,200 beta cells mixed with 25,600 non-beta or alpha cells. Then, cells were incubated at 37°C and 5% CO_2 for 5 days, and complete DMEM was changed after 48 h. These culture conditions allowed reaggregation of cells into pseudo-islets.

Insulin and glucagon secretion test in response to glucose by static incubation

After their formation, pseudo-islets were recovered within a tube, rinsed with complete DMEM containing 0.1% bovine serum albumin (BSA) (Sigma) instead of FBS and aliquots of 256 pseudo-islets in 1 mL of the same medium placed into cell culture inserts with pore size of 12 µm used in a 24-well plate. Under this condition, pseudo-islets were incubated overnight at 37°C and 5% CO_2. Before insulin secretion test, medium was replaced by same DMEM but supplemented with 2.8 mM glucose and preincubated 1 h at 37°C and 5% CO_2. Then, this medium was discarded and pseudo-islets were successively incubated 1 h in 600 µL DMEM with 2.8 mM glucose and 1 h in 600 µL DMEM with 16.7 mM glucose. Media (supernatants) were collected and protein content within granules extracted by incubating cells 1 h at room temperature in 600 µL acid ethanol solution (0.19 HCl in 75% ethanol). For glucagon secretion, a similar protocol was used, but pseudo-islets were preincubated at 11.2 mM glucose and incubated successively at 11.2, 5.6 and 1 mM glucose. Samples were stored at −20°C before measuring insulin and glucagon using ELISA kits (Mercodia, Uppsala, Sweden).

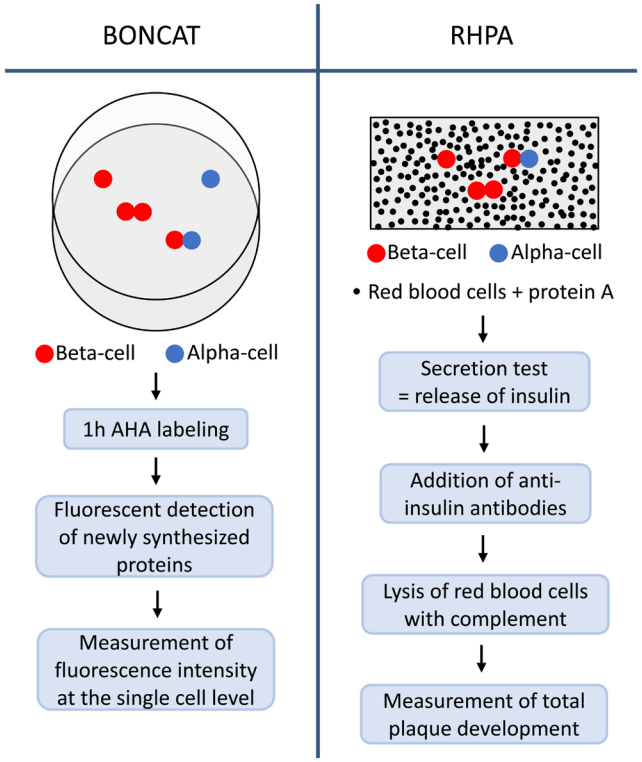

Figure 1
Schematic of the BONCAT strategy and of the RHPA method. The BONCAT strategy allows the labeling of newly synthesized proteins. Islet cells are exposed for 1 h to AHA, newly synthesized proteins are detected and visualized using an AF488-Alkyne, and fluorescence intensity is measured at the single cell level. The RHPA method allows the quantification of insulin secretion by beta cells at the single cell level. Briefly, islet cells are mixed with sheep red blood cells, a secretion test is performed to induce insulin release, anti-insulin antibodies are added to induce the lysis of red blood cells via the complement and the percentage of secreting cells and the plaque area are quantified.

Assessment of insulin secretion at single cell level

Insulin secretion of single and reaggregated beta cells was assessed by RHPA as previously described (Fig. 1) (Salomon & Meda 1986). Briefly, unsorted islet cells, after being incubated 24 h, were resuspended in Krebes–Ringer bicarbonate HEPES buffer (KRB) supplemented with 0.1% BSA (w/v), 2.8 mM glucose and 5% (v/v) of packed sheep red blood cells (Thermo Scientific) previously coated with protein A (Sigma) at a concentration of 300,000 cells/mL. Sixty microliter of this mixture were introduced into Cunningham chambers previously coated with 0.1 mg/mL poly-L-lysine (Sigma) and incubated at 37°C for 1 h to allow cells to attach. Then, chambers were submitted to the secretion phase. To this end, chambers were rinsed with KRB containing 2.8 mM or 16.7 mM glucose and incubated with the same buffer supplemented with a guinea pig polyclonal anti-insulin antibody (homemade diluted 1:300) at 37°C for 1 h. In one series of experiments, the secretion phase (in presence of anti-insulin antibody) lasted 20 min instead of 1 h. Then, chambers were rinsed with KRB and incubated 1 h at 37°C with KRB containing 2.8 mM glucose supplemented with a guinea pig complement (Sigma) diluted 1:40. Finally, chambers were rinsed with KRB, incubated 1 min with 0.04% (w/v) trypan blue solution in KRB, fixed with Bouin's solution (Sigma) and stored at 4°C in phosphate-buffered saline (PBS) supplemented with 0.1% azide (w/v) until immunofluorescence labeling.

AHA labeling of islet cells

Three-well glass slides (Thermo Scientific) were coated with 0.1 mg/mL poly-L-lysine (Sigma) at room temperature for 1 h, rinsed with water and air-dried. Aliquots of

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10^4 cells/50 µL in DMEM without methionine (Pan Biotech, Aidenbach, Germany), with 0.1% BSA and 2.8 or 11.2 mM glucose, depending on the experiments, were seeded on three-well glass slides and incubated for 1 h to allow cell attachment. Then, cells were exposed 1 h at 37°C to DMEM without methionine supplemented with 200 µM AHA (Fig. 1) (Click Chemistry ools, Scottsdale, AZ, USA). Depending on the experiments, different glucose concentrations were used, and 10 µM insulin (Sigma) or 10 nM glucagon (Sigma) were added as indicated in the 'Results' section. Islet cells were rinsed with PBS and fixed 20 min in 10% methanol-free formalin. Finally, islet cells were permeabilized with Triton X-100 for 15 min, washed in PBS and AHA labeling detected by incubating cells 30 min with 0.1 µM AF488-Alkyne (Jena Bioscience, Jena, Germany) diluted in a 'cell reaction buffer kit' (Click Chemistry Tools). Pictures were taken with a Leica DM 2000 microscope, using a 40× objective, for further analysis.

Simultaneous assessment of insulin secretion and AHA labeling at single cell level

Insulin secretion was assessed by RHPA as described above. In order to measure protein biosynthesis and insulin secretion on the same beta cells, 200 µM AHA was added either before or during the secretion phase. DMEM without methionine was used to rinse the cells and for the incubations instead of KRB. When analyzed, cells were divided into two categories depending on their AHA labeling intensity. Half of the cells with lower labeling were referred as 'Low AHA labeling' and the others with higher labeling were referred as 'High AHA labeling'.

Immunostaining of islet cells

After their labeling with AHA or after RHPA, islet cells were permeabilized with Triton X-100 for 15 min, washed in PBS, incubated 10 min in PBS supplemented with 0.1% BSA and then identified by immunofluorescence using specific antibodies. To this end, cells were exposed 2 h to one or a combination of the following specific antibodies: a guinea pig anti-insulin (Ventrex Laboratories, Portland, 1:300) and a mouse anti-glucagon (Sigma, 1:4000). Islet cells were rinsed and exposed 1 h to one or a combination of the following appropriate antibodies: AMCA donkey anti-guinea pig (Jackson, UK, 1:200), rhodamine goat anti-guinea pig (Jackson, 1:300) and AF594 donkey anti-mouse (Jackson, 1:1'200). Nucleus staining was performed using mountain medium with 4′,6-diamidino-2-phenylindole (DAPI) (Abcam, United Kingdom). All incubations were performed at room temperature and antibody dilutions in PBS supplemented with 0.1% BSA.

Analysis of biosynthetic labeling

Digital pictures were analyzed using the ImageJ Software. Each islet cell type, identified by immunofluorescence, was assessed for AHA labeling, which corresponds to the mean gray value (it is the sum of the gray values of all the pixels in the selection divided by the number of pixels). To this end, an area of the cytoplasm (always the same surface) was manually selected and fluorescence intensity of the AHA labeling in green was measured at the single cell level. A minimum of 30 and up to 100 islet cells were quantified for every condition.

Statistical analysis

Data are represented as means ± S.E.M. with each dot representing an experiment. Differences between means were assessed by the Student's paired *t*-test. All statistical analyses were performed with Prism software 9.3.1 (GraphPad), and $P < 0.05$ was considered statistically significant.

Results

Positive correlation between secretory and biosynthetic activities in single beta cells

Single beta cells were simultaneously studied for insulin secretion and protein biosynthesis by combined RHPA and AHA labeling test. Results of RHPA were analyzed separately for two groups of beta cells: the 50% with the lowest and the 50% with the highest AHA labeling. For both groups, we quantified by RHPA the percentage of plaque-forming cells (secreting-cells) and the mean hemolytic plaque area around secreting cells. Then these two values were multiplied together to obtain the total plaque development (TPD), which correspond to the total plaque area around 100 beta cells. As shown in Fig. 2A, beta cells with the highest AHA labeling had a 5.4- and 2.5-fold higher TPD in response to 2.8 or 16.7 mM glucose, respectively, compared to beta cells with the lowest AHA labeling. Then, we repeated the same experience, except that cells were labeled with AHA under resting conditions (0 or 2.8 mM glucose) for 1 h, before measuring insulin secretion in 16.7 mM glucose for 1 h. As shown in Fig. 2B,

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beta cells with the highest AHA labeling in 0 or 2.8 mM glucose had a 2.1- and 2.3-fold higher TPD compared to beta cells with the lowest AHA labeling, respectively. As shown in Fig. 2C, beta cells that have a low AHA labeling secrete less insulin in comparison with beta cells that have a high AHA labeling. Interestingly, we have also observed that the AHA labeling is heterogenous among beta cells, and that it is clearly higher in response to 16.7 mM compared to 2.8 mM glucose stimulation (Fig. 2D).

Different effects of beta-to-beta cell contacts on protein biosynthesis and insulin secretion

When compared to single beta cells, AHA labeling of beta cells in contact with another beta cell was about 1.3-fold higher at 2.8 and 11.2 mM glucose, but similar at 16.7 mM glucose (Fig. 3A and B). In parallel, when looking at the insulin secretion by RHPA, TPD of beta cell pairs in comparison to single beta cells was 2.4 and 2.2-fold higher in 11.2 and 16.7 mM glucose, respectively, but not significantly increased in 2.8 mM glucose (Fig. 3C and D). If incubated at 16.7 mM glucose for 20 min instead of 1 h, TPD of beta cell pairs displayed a high fold increase (3.5×) compared to single beta cells, showing that beta–beta contacts seem to be even more important during the first minutes of insulin secretion in response to glucose (Fig. 3E). However, under these conditions, beta-to-beta cell contacts did not affect AHA labeling (Fig. 3F).

Beta-to-alpha cell contacts do not affect beta cell protein biosynthesis and insulin secretion

When compared to single beta cells, beta cells in contact with an alpha cell showed similar AHA labeling. AHA labeling increased according to glucose similarly in single beta cells and beta cells in contact with an alpha cell. (Fig. 4A and B). In parallel, when looking at the insulin secretion by RHPA, TPD of beta–alpha cell pairs in comparison to single beta cells was unchanged at both 2.8 and 16.7 mM glucose (Fig. 4C and D). Similarly, alpha cells

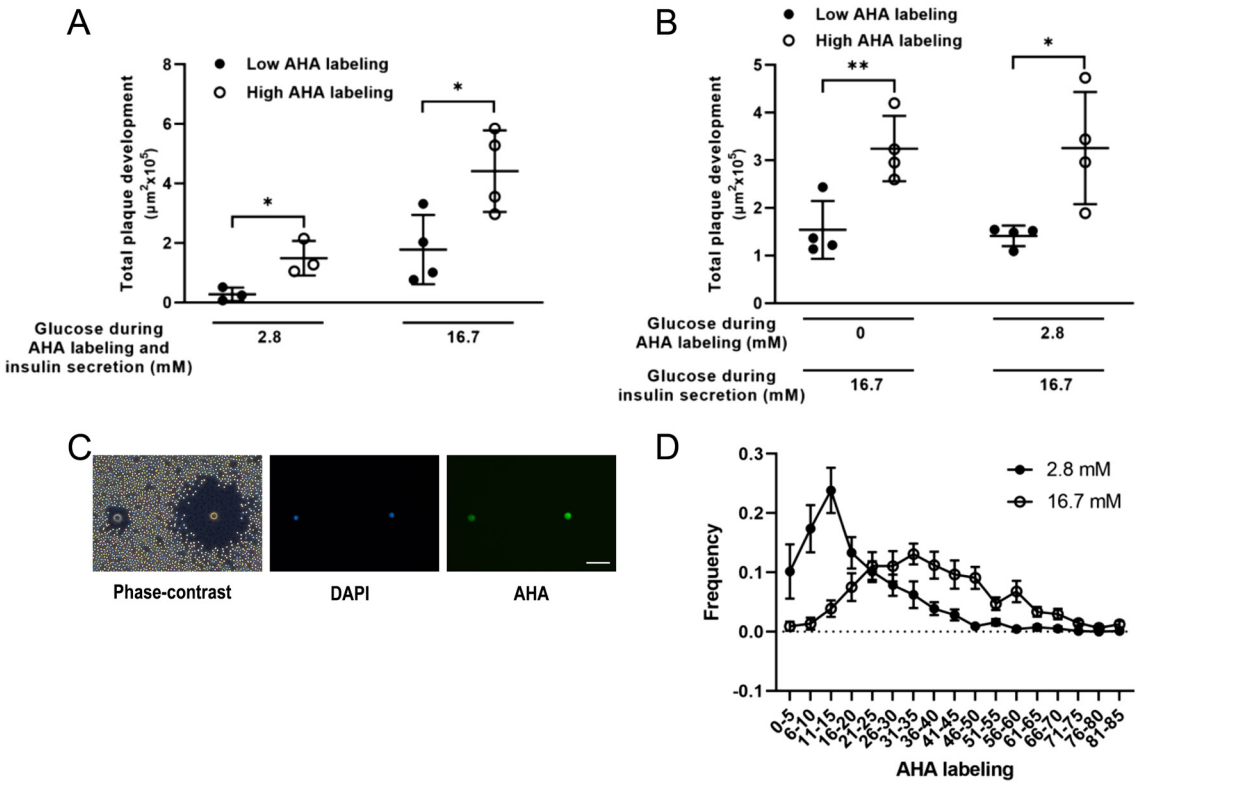

Figure 2
Positive correlation between insulin secretion and protein biosynthesis in single beta cells. (A) Total plaque development of single beta cells according to their low (black dots) or high (white dots) protein biosynthesis, when insulin secretion and biosynthesis were evaluated simultaneously in response to 2.8 mM or 16.7 mM glucose. (B) Total plaque development of single beta cells according to their low- (black dots) or high- (white dots) protein biosynthesis, when biosynthesis was evaluated before insulin secretion in response to 0 or 2.8 and 16.7 mM glucose, respectively. (C) Phase-contrast, DAPI staining (blue) and AHA labeling (green) of two beta cells at the end of the RHPA. The bar represents 50 µm. (D) Frequency of single beta cells according to their AHA labeling fluorescence intensity in response to 2.8 mM or 16.7 mM glucose stimulation ($n = 9$). *$P < 0.05$ and **$P < 0.01$.

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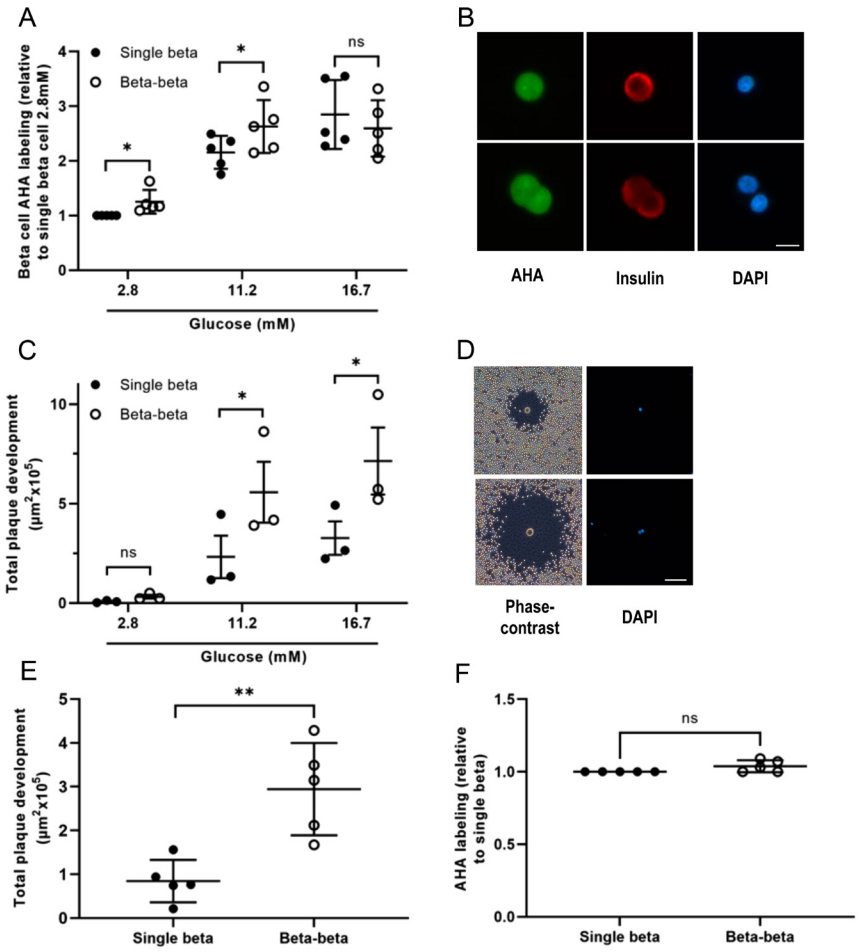

Figure 3
Beta cell–beta cell contact increases protein biosynthesis and insulin secretion. (A) AHA labeling of single beta cells (black dots) or two beta cells in contact (white dots) when stimulated 1 h with increasing glucose concentrations. (B). AHA labeling (green), insulin (red) and DAPI staining (blue) of a single beta cell and two beta cells in contact. The bar represents 10 µm. (C) Total plaque development of single beta cells (black dots) or two beta cells in contact (white dots) when stimulated 1 h with increasing glucose concentrations. D. Phase-contrast and DAPI staining (blue) of a single beta cell and two beta cells in contact at the end of the RHPA. The bar represents 50 µm. (E) Total plaque development of single beta cells (black dots) or beta cell pairs (white dots) when stimulated 20 min with 16.7 mM glucose. (D) AHA labeling of single beta cells (black dots) or beta cell pairs (white dots) when stimulated 20 min with 16.7 mM glucose. $^{*}P < 0.05$ and $^{**}P < 0.01$.

did not affect insulin secretion of beta cells when both cell types were reaggregated into pseudo-islets, and when insulin secretion was assessed by static incubation and measured by ELISA (Fig. 4E).

Protein biosynthesis of alpha cells is not affected by glucose but decreased when in contact with beta cells

Whereas glucose concentration-dependently increased AHA labeling in beta cells, it did not affect AHA labeling in alpha cells. At 2.8 mM glucose, AHA labeling in alpha cells was elevated compared to beta cells and did not increased anymore at higher glucose concentrations (11.2 and 16.7 mM) (Fig. 5A and C). Also, when compared to single alpha cells, AHA labeling of alpha cells in contact with a beta cell was similar at 2.8 but decreased at 11.2 and 16.7 mM glucose (Fig. 5B and C). In addition, glucagon secretion from alpha cells reaggregated into pseudo-islets was unaffected by glucose in absence of beta cells. By contrast, the presence of beta cells in pseudo-islets allowed alpha cells to increase glucagon secretion by 1.8-fold at lower glucose concentration (Fig. 5D). Regarding AHA labeling under these conditions, low glucose concentration had no effect on single alpha cell protein biosynthesis, but the presence of beta cells decreased alpha cell protein biosynthesis under 11.2, 5.6 and 1 mM glucose (Fig. 5E).

Insulin and glucagon do not affect beta and alpha cells protein biosynthesis

Since the main paracrine control of secretion in islets is through insulin and glucagon, we assessed whether these hormones were able to affect protein biosynthesis of alpha and beta cells. When compared to control, addition of 10 nM glucagon during AHA labeling test did not affect protein biosynthesis of beta cells (Fig. 6A), and addition of 10 µM insulin did not affect protein biosynthesis of alpha cells (Fig. 6B). However, when looking at the insulin secretion by RHPA, addition of 10 nM glucagon significantly increased the TPD of beta cells at least at 16.7 mM glucose (Fig. 6C).

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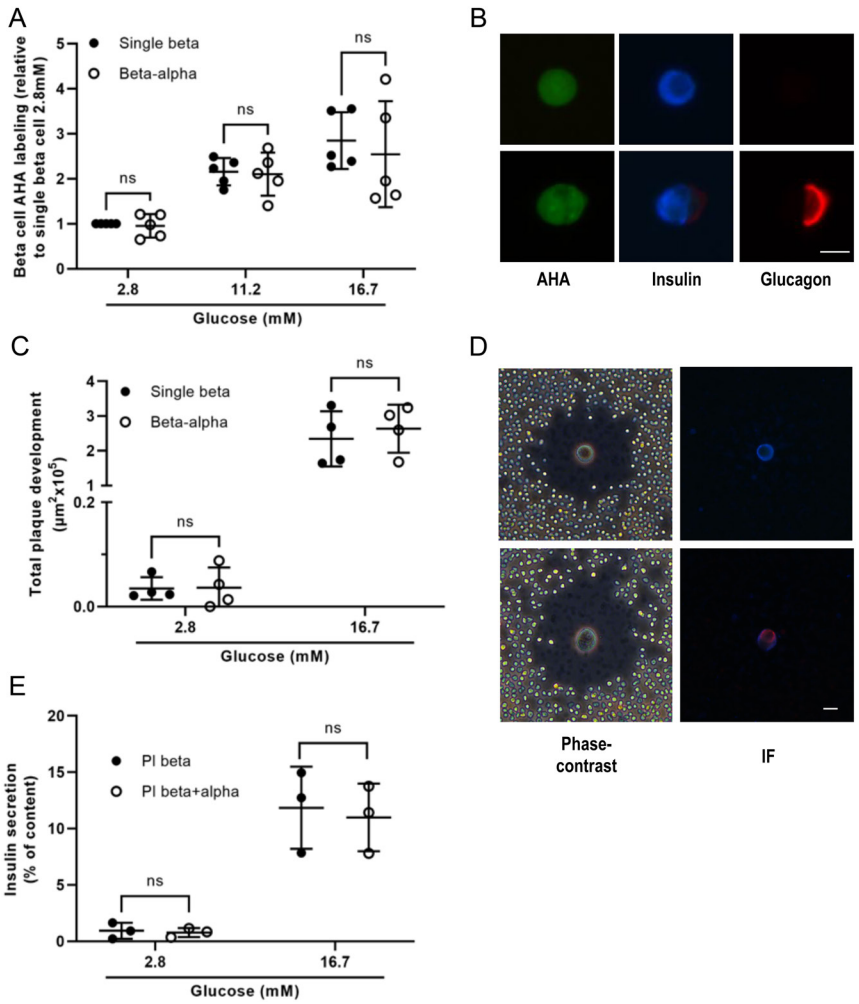

Figure 4
Beta cell–alpha cell contact does not affect beta cell protein biosynthesis and insulin secretion. (A) AHA labeling of single beta cells (black dots) or beta cells in contact with an alpha cell (white dots) in response to increasing glucose concentrations. (B) AHA labeling (green), insulin (blue) and glucagon (red) staining of a single beta cell and a beta cell in contact with an alpha cell. The bar represents 10 µm. (C). Total plaque development of single beta cells (black dots) or a beta cell in contact with an alpha cell (white dots) in response to 2.8 mM and 16.7 mM glucose. (D). Phase-contrast and DAPI staining (blue) of a single beta cell and a single beta cell and a beta cell in contact with an alpha cell at the end of the RHPA. The bar represents 10 µm. (E) Insulin secretion of pseudo-islets composed of beta cells (black dots) or beta and alpha cells (white dots) in response to 2.8 or 16.7 mM glucose.

Discussion

Pancreatic islets secrete various endocrine hormones including insulin and glucagon to regulate blood glucose level. In order to meet the changing metabolic need of the body, secretion of these hormones must be tightly regulated and islet endocrine cells must receive multiple regulatory signals. More recently, many studies emphasized the role of intercellular contacts, such as paracrine and juxtacrine interactions between islets cells on the secretion of insulin and glucagon. Mechanisms regulating stimulus–secretion coupling have been more extensively studied in beta cells, as the perturbation of insulin secretion is associated with diabetes. However, no studies investigated the effect of intercellular contacts on beta and alpha cell protein biosyntheses, even if it is evident that biosynthetic activity is essential in maintaining a correct function of islet cells.

In this study, we used the BONCAT strategy based on incorporation of an azide analog of methionine, AHA, which allows imaging and identification of nascent proteins in every individual cell. Importantly, this functional azide group is bioorthogonal, it does not cross react with natural biological chemistries (Calve *et al.* 2016). AHA labeling approach is a powerful method to study biosynthetic activity at the single-cell level and combined to immunofluorescence allows evaluating protein biosynthesis simultaneously in different islet cell types and according to their interactions with other islet cells. It allows also to study, in the same beta cell, protein biosynthesis by AHA labeling and insulin secretion by RHPA. In a previous work, we used a quite different approach based on incorporation of *O*-propargyl-puromycin (OPP) instead of AHA to label nascent proteins and showed the link between fluorescence intensity and protein biosynthesis (Cottet-Dumoulin *et al.* 2020). However, puromycin is an antibiotic protein synthesis inhibitor, which causes premature chain termination during translation. By substituting OPP with AHA, it is possible to label nascent proteins for a longer period without causing any deleterious effect on cells.

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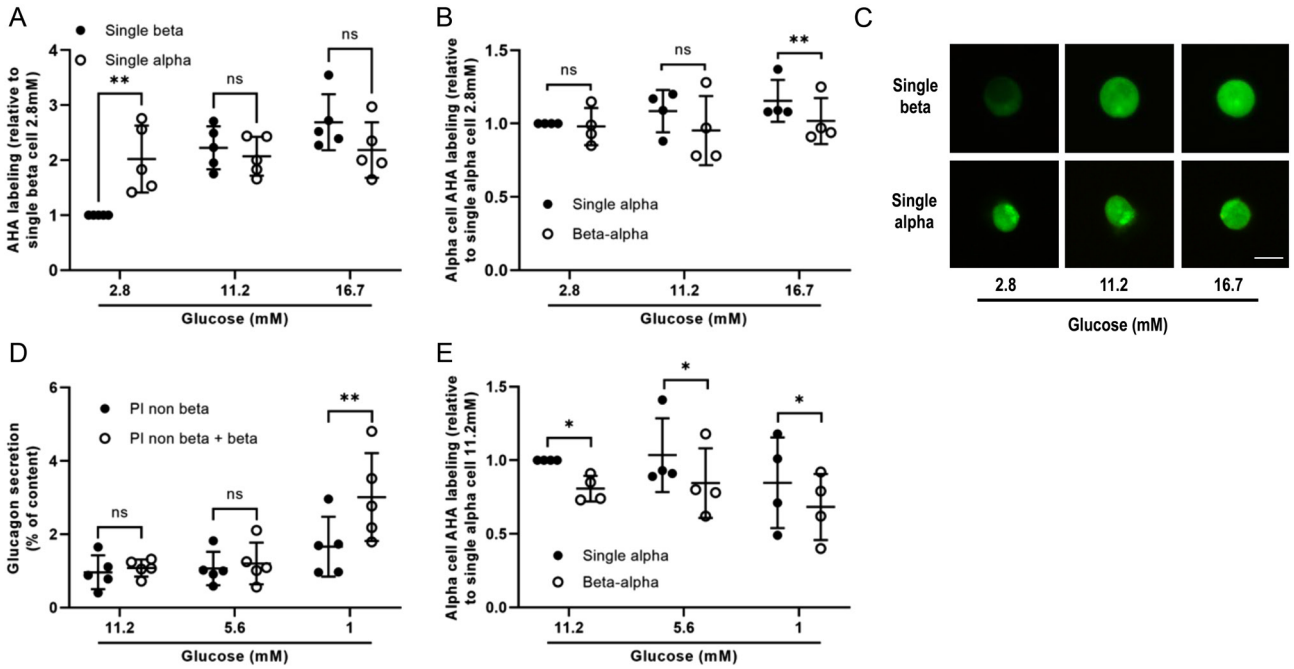

Figure 5

Alpha cell protein biosynthesis. A. AHA labeling of single beta cells (black dots) and single alpha cells (white dots) in response to increasing glucose concentrations. (B) AHA labeling of single alpha cells (black dots) and an alpha cell in contact with a beta cell (white dots) in response to increasing glucose concentrations. (C) AHA labeling (green) of single beta cells and single alpha cells in response to 2.8 mM, 11.2 mM and 16.7 mM glucose. The bar represents 10 µm. (D). Glucagon secretion of pseudo-islets composed of non-beta cells (black dots) or beta and non-beta cells (white dots) in response to decreasing glucose concentrations. (E) AHA labeling of single alpha cells (black dots) or an alpha cell in contact with a beta cell (white dots) in response to decreasing glucose concentrations. *$P < 0.05$ and **$P < 0.01$.

As expected, we observed that glucose, the main mediator of insulin secretion, increases protein biosynthesis of beta cells. In fact, it has been shown that glucose directly increases insulin biosynthesis of beta cells by stimulating insulin gene transcription, by stabilizing insulin messenger ribonucleic acid and by enhancing the speed of beta cell protein translation (Poitout *et al.* 2006, Vander Mierde *et al.* 2007). On the other hand, we showed that glucose had no effect on alpha cell protein biosynthesis. The effect of glucose on the biosynthesis of alpha cells was still unexplored, and even its effect on glucagon secretion is debated. Indeed, two studies showed a decrease of glucagon secretion at high glucose concentration via a decrease of cAMP levels in islets from C57BL/6 mice (Marchand & Piston 2012, Yu *et al.* 2019), while another study showed an increase of glucagon secretion at high glucose concentration without any modulation of cAMP levels in sorted alpha cells from Sprague–Dawley rats (Olsen *et al.* 2005). The stimulatory action of glucose in isolated alpha cells contrasts with the suppressive effect of the sugar in intact islets and highlights the primary importance of islet paracrine signaling in the regulation of glucagon release.

By combining RHPA and AHA labeling, we observed a positive correlation between the biosynthetic activity and insulin secretion in each individual beta cell. Indeed, beta cells with a high AHA labeling were secreting more insulin in response to glucose. Moreover, we showed that even before a secretion phase there was a biosynthetic activity heterogeneity among beta cells, and that beta cells with a high protein synthesis activity were more susceptible to secrete higher amount of insulin. Therefore, beta cells with a higher protein biosynthesis secrete more insulin. The reason for that is unknown. One possibility is that an increase of protein biosynthesis results in an increase of insulin content and consequently secreted insulin increases as well. Another possibility is that the effect of protein synthesis is on the machinery of insulin granule exocytosis or other steps involved in insulin secretion. Previous reports also demonstrated that newly synthesized insulin in beta cells is preferentially secreted compared to stored insulin (Halban 1982).

In line with previous studies, we showed that homologous contact between beta cells increased insulin secretion (Bosco *et al.* 1989, Brereton *et al.* 2006). Regarding protein biosynthesis, we observed that it was

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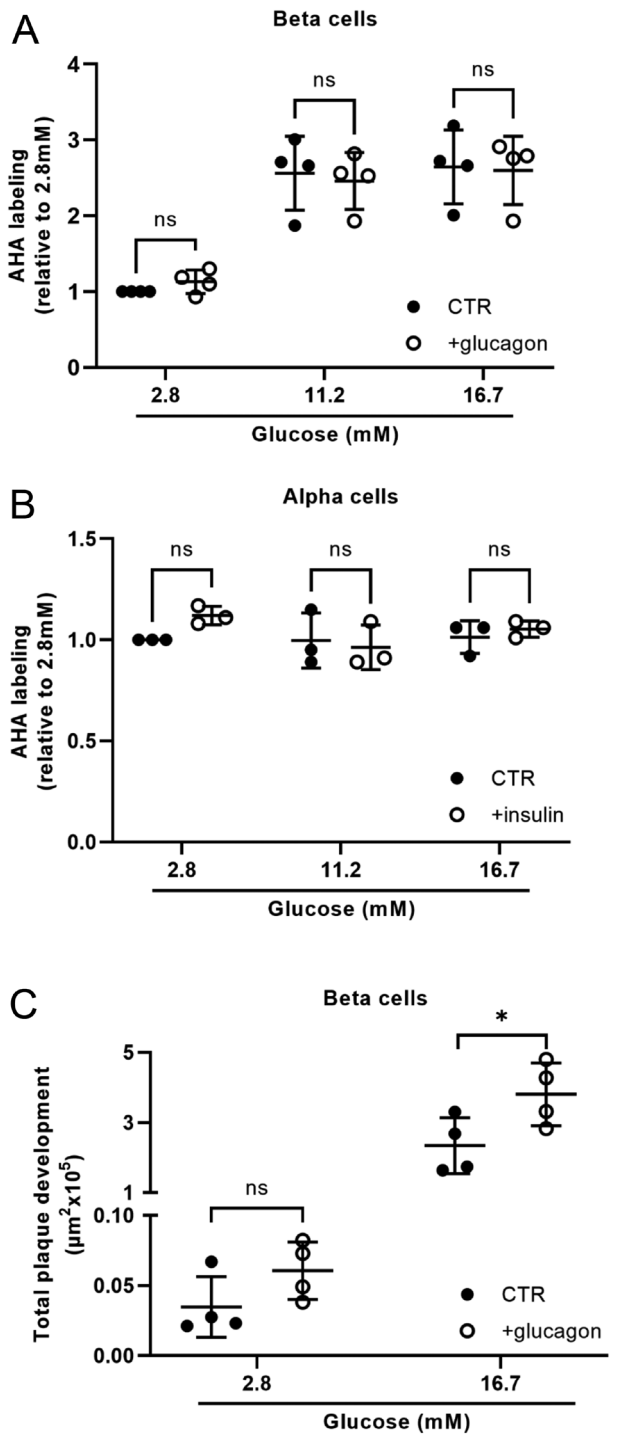

Figure 6
Effect of insulin and glucagon on beta and alpha cells protein biosynthesis and insulin secretion. (A) AHA labeling of single beta cells in control medium (black dots) or medium supplemented with glucagon (white dots) in response to increasing glucose concentrations. (B) AHA labeling of single alpha cells in control medium (black dots) or medium supplemented with insulin (white dots) in response to increasing glucose concentrations. (C) Total plaque development of single beta cells in control medium (black dots) or medium supplemented with glucagon (white dots) in response to 2.8 or 16.7 mM glucose. *$P < 0.05$.

increased due to homologous contacts at low but not high glucose concentration. Therefore, homologous contacts between beta cells seem to stimulate beta cell protein biosynthesis principally at low glucose levels, while they mainly stimulate insulin secretion at high glucose levels. In addition, we showed that the effect of direct contact between beta cells on insulin secretion was mainly seen at the beginning of the glucose stimulation. When stimulated only for 20 min with high glucose levels, insulin secretion of two beta cells in direct contact was much more increased compared to single beta cells and compared to 1 h glucose stimulation. However, even under those conditions, beta cell protein biosynthesis was not affected.

We showed that heterologous contacts between beta and alpha cells did not affect beta cell insulin secretion and protein biosynthesis. The fact that alpha cells do not influence insulin secretion from beta cells through cell-to-cell contacts is consistent with previous studies (Bosco *et al.* 1989, Brereton *et al.* 2007). Unlike human islets where most beta cells are in contact with alpha cells, rodent islets are composed of a central core of beta cells and peripheral alpha cells. It has been shown in human islets that direct contact between alpha and beta cells increases insulin secretion (Wojtusciszyn *et al.* 2008). In rodents, beta cells are mostly in contact other beta cells, and direct contact between beta and alpha cells are rare (Cabrera *et al.* 2006). Consequently, it is more likely that alpha cells affect beta cell function via paracrine and/or juxtacrine interactions in human islets than in rat islets. Nevertheless, beta and alpha cells could affect each other in rodents via interstitial or vascular routes (Samols & Stagner 1988, 1990, Kim *et al.* 2009). It should be noted that the rodent core-mantle islet architecture has recently been debated and might be more complex than previously described. Indeed, it has been shown that a complete mantle of non-beta cells would require a higher proportion of non-beta cells in the islet compared to what has been observed up to now. As for the islet vasculature, it has mostly been studied independently from that of the exocrine pancreas, while the blood flow between endocrine and exocrine pancreas might play an important role (Dybala *et al.* 2020). In this work, we showed that direct contact of an alpha cell with a beta cell decreased alpha cell protein biosynthesis. This effect was observed under different glucose concentrations. Interestingly, it was previously reported that the αTC1.9 cells (alpha cell line), when in contact with MIN6 cells (beta cell line), had a reduced glucagon content (Kelly *et al.* 2010). Nowadays, there is no possibility to study glucagon secretion at the single cell level as for instance by

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RHPA. However, glucagon secretion of pseudo-islets can be assessed by combining static incubation and ELISA, and we showed that the presence of beta cells was important for glucagon secretion at low glucose concentration. Indeed, pseudo-islets composed of non-beta cells (mainly alpha cells) and beta cells, compared to pseudo-islets composed of non-beta cells only, showed a significantly higher glucagon secretion in response to 1 mM glucose. This indicates that the presence of beta cells is required for a correct glucagon secretion from alpha cells, even if there was no effect on protein biosynthesis. Whether this is because of juxtacrine or paracrine effect via insulin for instance remain to be understood. Another explanation could be that addition of beta cells within non-beta cell pseudo-islets reduces the relative number of delta cells and the known effects of somatostatin to inhibit glucagon secretion (Strowski *et al.* 2000).

In this study, we focused on direct cell-to-cell contacts as intercellular junctions have been shown to be critical for islet hormone regulation. Indeed, cell adhesion molecules such as cadherins, Eph receptors or ephrin ligands, and gap junctions can mediate cell-to-cell interactions. E-caherin has been shown to be essential for glucose-stimulated insulin secretion (GSIS) and its downregulation leads to defects in glucose-stimulated synchronicity of calcium oscillations between regions of the islets (Rogers *et al.* 2007, Jaques *et al.* 2008, Parnaud *et al.* 2015). Gap junctions, composed of connexins, are known to be involved in beta cell synchronization of their activities by mediating the rapid exchange of small molecules. Knockdown of the most expressed connexin in islets, Cx36, leads to disrupted intracellular Ca^{2+} concentration and loss of cell-to-cell synchronization (Serre-Beinier *et al.* 2000, Ravier *et al.* 2005). Regarding the Eph/ephrin signaling system, it has been shown that EphA forward signaling inhibits insulin secretion, whereas ephrin-A reverse signaling stimulates insulin secretion (Konstantinova *et al.* 2007). There are also evidences that stimulation of alpha cells EphA receptors by beta-cell ephrin ligands, probably via an increase of RhoA activity, can negatively regulate glucagon secretion (Hutchens & Piston 2015, Hughes *et al.* 2018, Ng *et al.* 2022). Nonetheless, the effect of secreted hormones, mainly insulin and glucagon, via paracrine contacts, cannot be excluded in our experiments. We hypothesized that insulin and glucagon, the main hormones secreted by beta and alpha cells, respectively, could be responsible of paracrine effects observed within islets. As previously demonstrated, we showed that glucagon increased beta cell insulin secretion (Samols *et al.* 1965, Song *et al.* 2017). However, we did not observe any effect on beta cell protein biosynthesis. Finally, when looking at alpha cell protein biosynthesis, addition of insulin had no effect.

Taken together, our results emphasize the role of paracrine factors and direct cell-to-cell contacts for the function of islet cells. We showed that beta-beta contacts increased insulin secretion and protein biosynthesis. When looking at beta-alpha contacts, they had no effect on beta cell insulin secretion and protein biosynthesis. However, they increased glucagon secretion at low glucose concentration and decreased alpha cell protein biosynthesis. Even if beta cell insulin secretion was increased in response to glucagon, it was not the case for protein biosynthesis, and alpha cell protein biosynthesis was not affected by insulin. Finally, it appears that islet cells with a high biosynthetic activity are more susceptible to respond to external stimuli such as glucose levels.

Declaration of interest
No conflict of interest.

Funding
This work was supported by grants from the Swiss National Science Foundation (Grant #31003A_170090).

Author contribution statement
DC and DB conceived the study. DC, DM, QP and VL performed the experiments and finalized the dataset. DC, QP, VL, DM, LF, JB, RH, GP, FL, KB, EB, TB and DB contributed to the data analysis and discussion. DC and DB drafted the manuscript and all authors revised and edited the manuscript. All authors read and approved the final manuscript. DC and DB are the guarantors of this work taking responsibility for the integrity of the data and the accuracy of the data analysis.

Data availability
All data generated or analyzed during this study are included in this published article.

Acknowledgements
The authors thank Corinne Sinigaglia, Caroline Rouget and Nadine Pernin for excellent technical assistance.

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Received 24 May 2023
Accepted 30 May 2023
Available online 31 May 2023
Version of Record published 11 July 2023