Asymmetrical distribution of δ and PP cells in human pancreatic islets

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Abstract

The aim of this study was to evaluate the location of PP and δ cells in relation to the vascularization within human pancreatic islets. To this end, pancreas sections were analysed by immunofluorescence using antibodies against endocrine islet and endothelial cells. Staining in different islet areas corresponding to islet cells adjacent or not to peripheral or central vascular channels was quantified by computerized morphometry. As results, α, PP and δ cells were preferentially found adjacent to vessels. In contrast to α cells, which were evenly distributed between islet periphery and intraislet vascular channels, PP and δ cells had asymmetric and opposite distributions: PP staining was higher and somatostatin staining was lower in the islet periphery than in the area around intraislet vascular channels. Additionally, frequencies of PP and δ cells were negatively correlated in the islets. No difference was observed between islets from the head and the tail of the pancreas, and from type 2 diabetic and non-diabetic donors. In conclusion, the distribution of δ cells differs from that of PP cells in human islets, suggesting that vessels at the periphery and at the centre of islets drain different hormonal cocktails.

Key Words
- pancreatic islets
- architecture
- PP cells
- δ cells

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Introduction

Islets of Langerhans comprise different endocrine cell types. β cells secreting insulin and α cells secreting glucagon are the main islet cell types in most animal species, including human, and exhibit a clear and established function in glucose homeostasis. These two hormones have diametrically opposite actions on glucose regulation, and consequently, the secretion of one hormone is controlled by the other one. δ cells secreting somatostatin and PP cells secreting pancreatic polypeptide are less abundant (Bosco *et al*. 2005), and less attention has been paid to their function in metabolism. Nevertheless, somatostatin has been shown to inhibit both insulin and glucagon secretions (Schuit *et al*. 1989) and thus participates, albeit indirectly, in glucose homeostasis. Pancreatic polypeptide is mostly involved in satiety (Batterham *et al*. 2003) and in the regulation of pancreatic exocrine secretion (Putnam *et al*. 1989, Morisset 2008), but is known to exhibit some glucose regulatory function as well, for instance by modulating insulin receptor expression in hepatocytes (Seymour *et al*. 1996) and inhibiting glucagon release in mouse pancreatic islets (Aragon *et al*. 2015). It is generally accepted that local interactions between the different islet hormones play fundamental roles in the islet physiology, and some definite interactions are facilitated by the specific positioning of cell types within the islets (Hauge-Evans *et al*. 2009, Unger & Orci 2010, Rodriguez-Diaz *et al*. 2011, Koh *et al*. 2012).

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A prototypical islet description was conducted from studies in rodents showing a relatively simple islet organization with a core of β cells surrounded by a mantle of other cell types. It has been proposed that this specific architecture is functionally relevant. Indeed, taking into account the microvascularization and the direction of the blood flow within the islet, one can conclude that a given secreted hormone (e.g., insulin) may affect the secretion of another one (e.g., glucagon) within the same islet (Bonner-Weir & Orci 1982, Marks *et al*. 1990). In humans, islets have a more complex cell organization and exhibit heterogeneous cell composition that furthermore varies according to their location into the pancreas (Stefan *et al*. 1982, Kim *et al*. 2009, Bosco *et al*. 2010, Wang *et al*. 2013, Bonner-Weir *et al*. 2015). Whereas smaller human islets have a core–mantle structure quite similar to that of rodent islets, the larger human islets have a more complex organization with endocrine cells organized into a continuous folded epithelial band (Bosco *et al*. 2010), evoking fused or even discrete core–mantle subunits in pancreas sections. For reasons of simplification, we hereafter use the term "subunit" to refer to a cross section through folded epithelial band.

In human pancreas, blood vessels are adjacent to the periphery of islets and others are found to invaginate into the central part and transit through vascular channels between the subunits in larger islets. Therefore, the mantle cells in subunit clusters are adjacent either to peripheral or to invaginating vessels (Bosco *et al*. 2010). The blood flow in human islets is so complex that a complete understanding of the microcirculation is lacking. In addition, it is not known whether the vessels at the periphery and those inside the islets originate from a common or different vessel ramification (Brunicardi *et al*. 1996, Ballian & Brunicardi 2007). Consequently, whether and how microcirculation affects the function of islet cell types is not understood either. Variations in islet cell composition, mostly related to α and PP cells, have been shown between ventral and dorsal parts of the human pancreas (Orci *et al*. 1976, Baetens *et al*. 1979, Stefan *et al*. 1982). Interestingly, these two pancreas parts are known to be irrigated by two arterial systems (Orci *et al*. 1976, Bertelli *et al*. 1997), suggesting that the nonhomogeneous distribution of cell types in islets may have functional importance. Whether cell distribution is similar between subunit mantle regions adjacent to the peripheral and invaginating vessels has not yet been investigated. In this work, we investigated the distribution of α, β, δ and PP cells by immunofluorescence on pancreas sections according to three different areas of the islets: the periphery (adjacent to peripheral vessels), the area adjacent to intraislet vascular channels and the area distant from periphery and intraislet vascular channels (the core of subunits). We also analysed the relative frequencies of PP and δ cells in the very same islets and studied whether the distributions of PP and δ cells differed between islets from the head and the tail of the pancreas, and from type 2 diabetic and non-diabetic donors.

Materials and methods

Pancreas procurement

Human pancreata were harvested from adult heart-beating brain-dead donors and designed to be processed for islet isolation and transplantation. Within the last 15 years, for diverse adverse motives (prolonged time for ischaemia, suspicion of tumours, type 2 diabetes, unforeseen technical difficulties, etc.), some pancreata were not processed for islet isolation and small specimens were taken for histology before organ destruction. Specimens from 25 donors were used for these analyses. Of these specimens, 18 were from non-diabetic donors with a mean age of 45 ± 16 years (range 15–69 years) and a mean body mass index (BMI) of 25 ± 4 (range 20–34). Specimens from non-diabetic donors were obtained from the head ($n=3$), body ($n=2$), tail ($n=4$) and unspecified regions ($n=8$) of the pancreas. For two pancreata, specimens from both the head and the tail were available. Specimens from type 2 diabetic donors were obtained from the head ($n=2$), body ($n=2$), tail ($n=4$) and unspecified regions ($n=3$) of the pancreas. Type 2 diabetic donors had a mean age of 58 ± 11 years (range 26–65 years) and a BMI of 31 ± 8 (range 26–51). Glycaemia at the time of hospital admission was 7.5 ± 3.7 mmol/L (mean ± S.D., $n=13$) for non-diabetic donors and 14.4 ± 6.2 mmol/L (mean ± S.D., $n=6$) for type 2 diabetic donors. Some control and diabetic specimens were the same used in a precedent work (Bosco *et al*. 2010). Histopathological analysis of the diabetic specimens revealed that several β cells were positive for amylin (Bosco *et al*. 2010).

Histological sections of pancreata

Pancreas samples were incubated for 24 h in 10% neutral buffered formalin, then dehydrated and embedded in paraffin before being sectioned at 5 µm. For each pancreas sample, one to three sections, separated by 500 µm, were randomly performed in order to analyse at least four islets per pancreas sample. All islets on the pancreas

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sections that fit with the requirements (presence of at least one vascular channel, one δ cell, or one PP cell) were analysed.

Immunofluorescence

Sections were deparaffinized and rehydrated with a series of alcohol solutions of decreasing concentrations. Sections were then washed with phosphate buffer saline (PBS) and treated for 10 min at 37°C with a 0.1% trypsin solution. They were washed again with PBS and successively treated for 20 min with 0.5% Triton X100 in PBS and 20 min with 0.1% BSA in PBS at room temperature. Then, the sections were incubated at room temperature with antibodies diluted in PBS as follows. Sections were first incubated for 1h with a mouse anti-CD34 antibody (AbD; Serotec, Bio-Rad), diluted 1:20, washed and incubated with an Alexa 488-conjugated anti-mouse antibody (1:500; Invitrogen). After washing, the sections were incubated for 1 h with a guinea pig anti-insulin antibody (1:300; Dako), together with one of the following rabbit antibodies: anti-glucagon, diluted 1:100, anti-somatostatin, diluted 1:200 and anti-PP, diluted 1:200. After washing, the sections were incubated for 1h simultaneously with aminimethylcoumarin-conjugated anti-guinea pig antibody, diluted 1:400 and rhodamine-conjugated anti-rabbit antibody, diluted 1:200 (Jackson ImmunoResearch Laboratories).

Morphological analysis and quantifications

Microscopic sections were analysed using a Leica DM2000 microscope (Leica Microsystems) equipped with ultraviolet illumination and filters for blue, red and green fluorescences. Images were captured using a Leica digital camera (Leica Microsystems) and recorded on computer through the software (Leica Microsystems). Quantifications of stained areas were performed on digital images using the Metamorph imaging software for microscopy (Universal Imaging, West Chester, PA, USA). This software was programmed to automatically quantify stained areas within defined regions of interest. Insulin, glucagon, somatostatin and PP-stained areas were measured in islets comprising at least one intraislet vascular channel identified by CD34 staining. The islets displaying no vascular channel, which were excluded from the analysis, were mostly with a diameter of about 50 µm or smaller (Bosco *et al.* 2010). Their frequencies were very variable between the pancreata, but did not exceed 20% of all islets. In each analysed islets, three regions were defined: peripheral region, the region around intraislet vascular channels and the core of islet subunits. The islet peripheral region was defined as the most outer layer of the cells, adjacent to islet surrounding blood vessels. Thus, a demarcation line was drawn around and 15 µm distant from the outer islet border. The area around intraislet vascular channels was defined as one layer of cells adjacent to islet invaginating vessels. Thus, a demarcation line was drawn around and 15 µm distant from the border of intraislet vascular channels. The remaining islet area (beyond the 15 µm demarcation lines) was considered as the core of islet subunits. In each region, pixel area occupied by glucagon, somatostatin, or PP staining was measured (pixel area A) and expressed relative to that of insulin staining (pixel area B) by the formula 100× pixel area A/(pixel area A + pixel area B). To study the relative frequencies of δ and PP cells in the same islets, pixel areas labelled for somatostatin or PP staining (using different fluorochromes) were measured in islets displaying at least one δ or PP cell. Pixel areas are then expressed relative to the pixels of the whole islet area measured manually by drawing the contour of the islets. Data were expressed as the mean ± S.E.M. of islets (from all pancreata) or the means of islets by pancreata. Differences between means were assessed either by the Student's *t*-test or, when required, by one-way ANOVA. When ANOVA was applied, Fisher's least significant difference *post hoc* analysis was used to identify significant differences.

Results

The aim of this study is to compare islet cell distribution between peripheral and invaginating vessels; therefore, only islets presenting at least one recognizable intraislet vascular channel throughout pancreas sections were analysed. We first analysed islets from random sections of the pancreas of non-diabetic donors. In human islets, endocrine cells are organized into a continuous trilaminar folded epithelial band, which looks like discrete subunits in cross section (Bosco *et al.* 2010). As previously shown, α cells were more frequently found at the periphery of these subunits, and β cells were mainly present in the core of these subunits, i.e., at least one cell diameter distant from blood vessels (Bosco *et al.* 2010). Similarities and differences in topographical distributions were observed for δ and PP cells. Similar to α cells, δ and PP cells were rare in the core of islet subunits and were more often observed at their periphery (Fig. 1A, B, C, D, E, and F). Interestingly, unlike α cells, δ and PP cells were not symmetrically distributed between the intraislet vascular channels and

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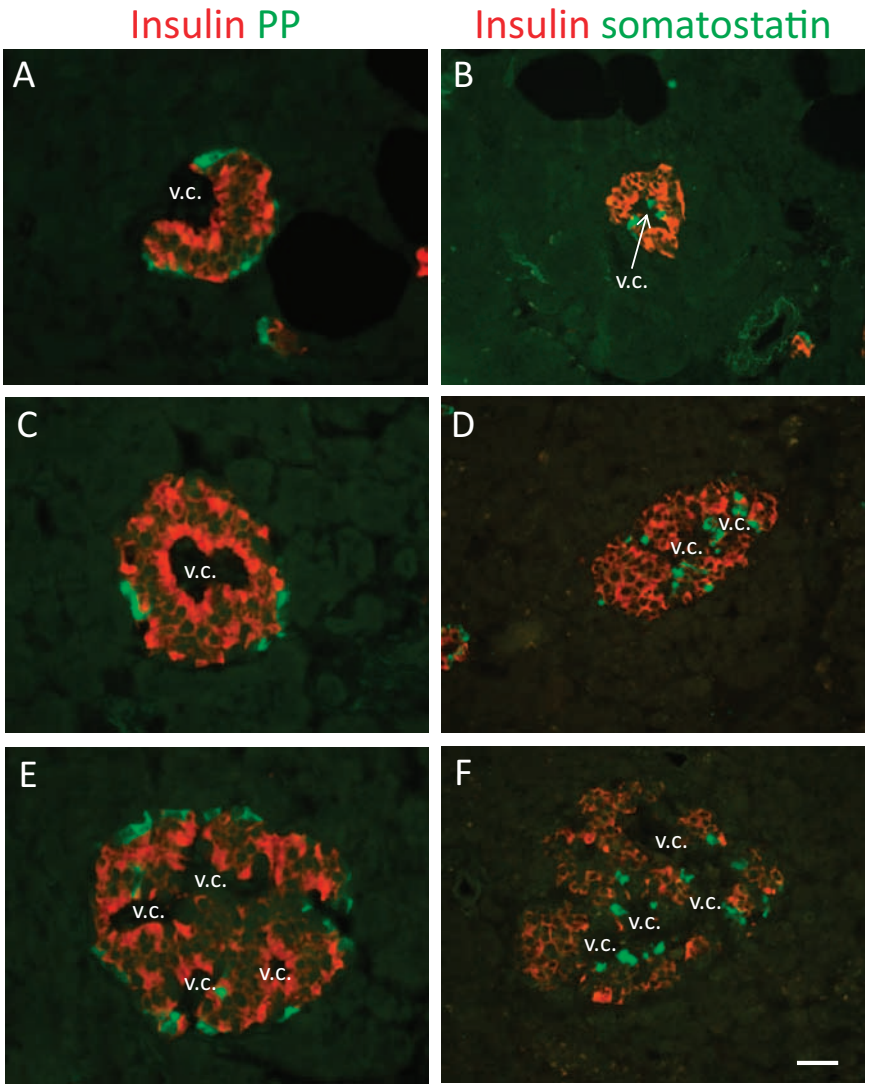

Figure 1
Different locations of PP and δ cells in human pancreatic islets. Pancreas sections were double labelled either for PP and insulin (A, C and E) or for somatostatin and insulin (B, D and F). (A, C and E) PP staining was mostly observed at the islet periphery compared with the area around intraislet vascular channels (v.c.). (B, D and F) Somatostatin was mostly observed at the area around (v.c.) compared with the islet periphery. Similar distribution patterns were observed in small (50–100 μm diameter, A and B), medium-sized (100–200 μm diameter, C and D) and bigger islets (> 200 μm diameter, E and F). Scale bar: 25 μm in A, C and E; 40 μm in B, D and F.

the islet periphery: δ cells were more frequently found adjacent to the intraislet vascular channels (Fig. 1B, D, and F) whereas PP cells were more frequently found in the islet periphery (Fig. 1A, C, and E). No difference in cell organization was observed according to the size of islets (Fig. 1A, B, C, D, E, and F).

To confirm differential islet cell distribution between α, δ and PP cells, immunofluorescence staining on digital images of pancreatic islets was quantitatively analysed by computer-assisted morphometry. To minimize the effect of islet size on results, areas stained for glucagon, somatostatin and PP were expressed relative to the area stained for insulin (hereafter referred as stained area ratio). We found that the stained area ratio for glucagon was similar between the islet periphery and the region around intraislet vascular channels and higher in the core subunit region (Fig. 2A). These results confirm our previous work reporting that human islet cells are organized into trilaminar epithelial bands with a symmetrical α cell distribution (Bosco *et al.* 2010). The somatostatin-stained area ratio was higher in the region around the intraislet vascular channel than in the peripheral or core regions (Fig. 2C). The somatostatin-stained area ratio in the peripheral region was similar to that in the core subunit region (Fig. 2C), which mainly contained β cells, confirming that a limited amount of δ cells was present at the peripheral region. On the contrary to what observed for somatostatin, the stained area ratio for PP was higher in the peripheral region than around the intraislet vascular channel region (Fig. 2B). The PP-stained area ratio in the area around the intraislet vascular channel was slightly higher than that in the core subunit region (Fig. 2B), further confirming that limited amount of PP was present around the intraislet vascular channel region.

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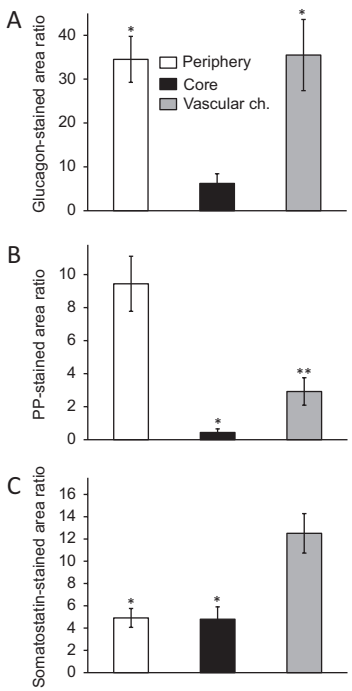

Figure 2
Quantification of islet cell locations in human pancreatic islets. Stained area ratios for glucagon (A), PP (B) and somatostatin (C) are shown according to the islet peripheral (white columns), core (black columns) and vascular channel (vascular ch.; grey columns) regions. (A) Glucagon-stained area ratios are similar between the islet periphery and the area around intraislet vascular channels and are higher (*P=0.004) than in the core of islet subunits; n=5 pancreata for a total of 18 islets. (B) PP-stained area ratio is higher in the islet periphery compared with the core of islet subunits (*P<0.0001) and the area around intraislet vascular channels (**P=0.02); n=12 pancreata for a total of 55 islets. (C) Somatostatin-stained area ratio is higher (*P < 0.0001) in the area around the intraislet vascular channels than in the islet periphery and the core of islet subunits; n=14 pancreata for a total of 83 islets.

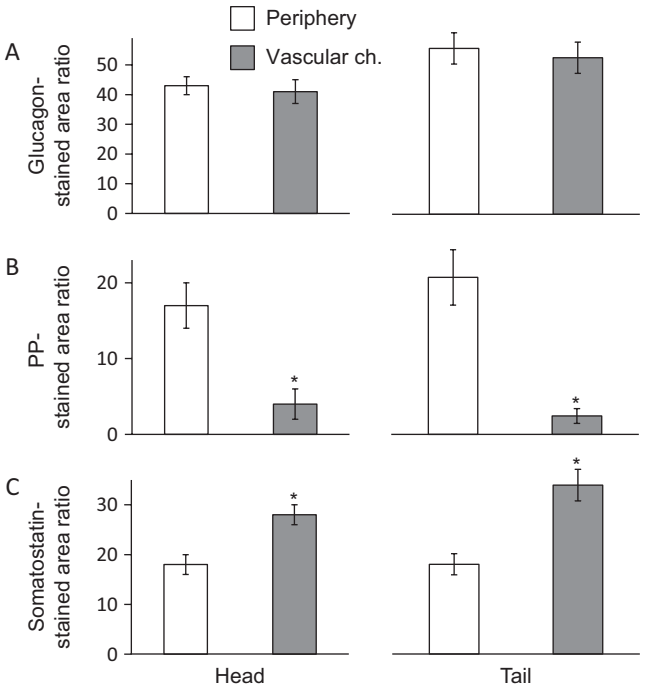

Figure 3
Similar islet cell locations in islets from the tail and the head of pancreas. Stained area ratios for glucagon (A), PP (B) and somatostatin (C) are shown according to the islet peripheral (white columns) and vascular channel (vascular ch.; grey columns) regions in the head and tail pancreas regions. (A) Glucagon-stained area ratios are similar between the islet periphery and the area around intraislet vascular channels, and this pattern is similar between the islets from the head (n=38 islets from four pancreata) and the tail of the pancreas (n=34 islets from four pancreata). (B) PP-stained area ratio was lower (*P<0.001) in the area around the intraislet vascular channels than the islet periphery, and this pattern is similar between the islets from the head (n=28 islets from four pancreata) and the tail of the pancreas (n=26 islets from four pancreata). (C) Somatostatin-stained area ratio was higher (*p<0.05) in the area around the intraislet vascular channels than the islet periphery, and this pattern is similar between the islets from the head (n=41 islets from four pancreata) and the tail of the pancreas (n=41 islets from four pancreata). When compared with the results in Fig. 2, values here are higher; this is due to a lower staining for insulin (taken into account in the calculation of the stained area ratio) in this series of experiments.

These results were not biased by the fact that stained areas in each islet regions were expressed relative to the area stained for insulin. Indeed, similar results were obtained when stained areas were expressed relative to the whole areas instead of the insulin-stained areas (Supplementary Fig. 1, see section on supplementary data given at the end of this article). This similarity is further rationalized by the relative weak variations in insulin staining observed between the different islet regions (periphery, core and vascular channel) (Supplementary Fig. 2).

As some variations in islet cell composition have been reported between different regions of the pancreas, islets from the tail and head regions of the pancreas were compared in terms of δ and PP distributions. The pancreatic uncinate processes with PP cell-rich islets were excluded from the analysis. As shown in Fig. 3A, B, and C, distributions of α, PP and δ cells around the intraislet vascular channel and the peripheral regions of islets were similar between the head and the tail of the pancreas.

δ and PP cell distributions were also analysed in pancreatic islets from type 2 diabetic donors. As control, pancreas sections from non-diabetic donors were additionally and concomitantly processed by immunofluorescence to minimalize the impact of technical variations of staining on the results. As shown in Fig. 4A, B, and C, distributions of α, PP and δ cells around the intraislet vascular channel and the peripheral regions of islets were similar in islets from non-diabetic and type

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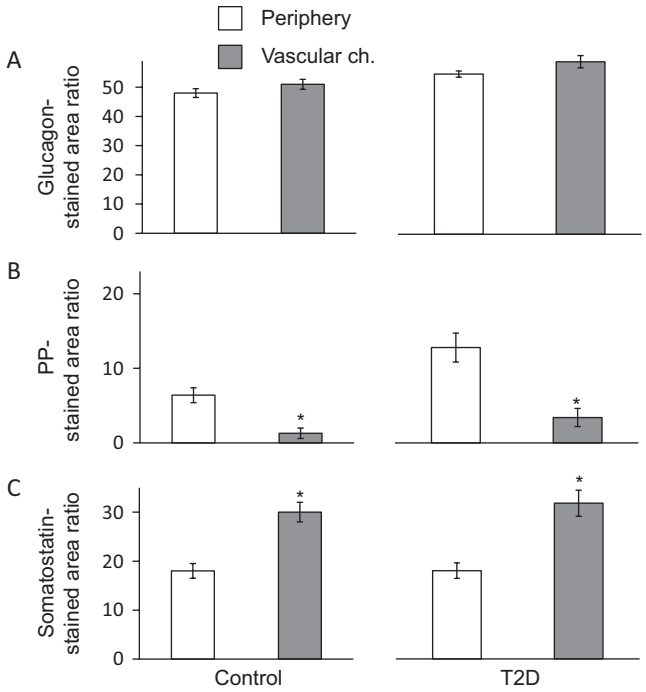

Figure 4
Similar islet cell locations in islets from non-diabetic and T2D donors. Stained area ratios for glucagon (A), PP (B) and somatostatin (C) are shown according to the islet peripheral (white columns) and vascular channel (vascular ch.; grey columns) regions, in non-diabetes (Control) and type 2 diabetes (T2D) donors. (A) Glucagon-stained area ratios are similar between the islet periphery and the area around intraislet vascular channels, and this pattern is similar between the islets from non-diabetic (control) donors ($n=147$ islets from 11 pancreata) and T2D donors ($n=189$ islets from five pancreata); these data are obtained from analyses previously made in (Bosco *et al*. 2010). (B) PP-stained area ratio is lower (*$P<0.001$) in the area around the intraislet vascular channels than the islet periphery, and this pattern is similar between the islets from non-diabetic (control) donors ($n=31$ islets from six pancreata) and T2D donors ($n=28$ islets from five pancreata). (C) Somatostatin-stained area ratio is higher (*$P<0.01$) in the area around the intraislet vascular channels than the islet periphery, and this pattern is similar between the islets from non-diabetic (control) donors ($n=83$ islets from four pancreata) and T2D donors ($n=59$ islets from nine pancreata).

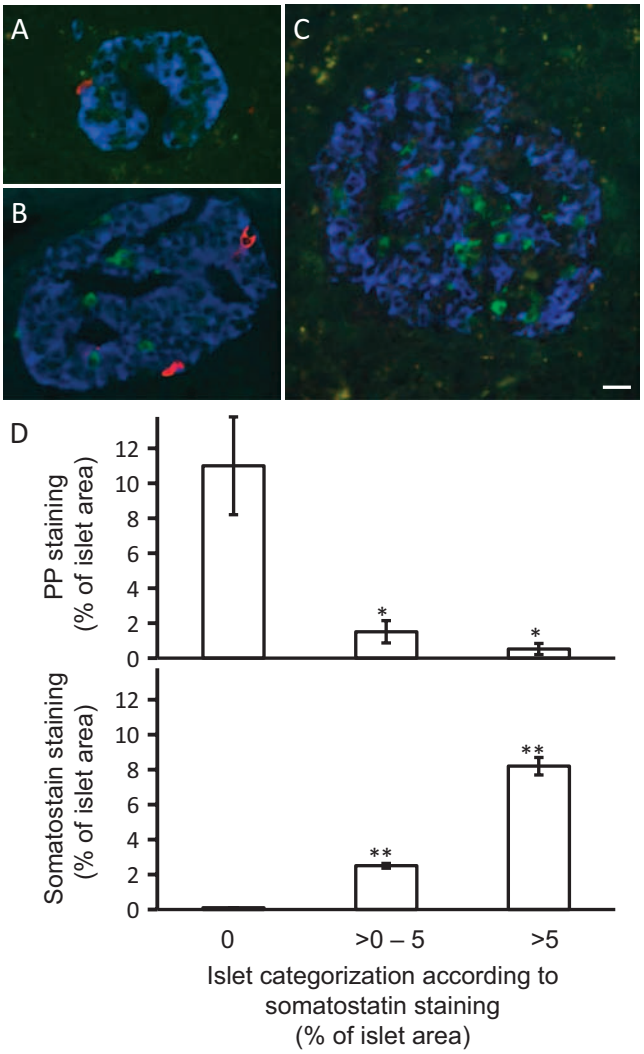

Figure 5
Non-concomitant occurrences of PP and δ cells in islets. (A, B and C) Islet sections were triple labelled for PP (red), somatostatin (green) and insulin (blue). (A) One islet displaying insulin (blue) and PP staining (red). (B) One islet displaying insulin (blue), PP (red) and somatostatin staining (green). (C) One islet displaying insulin (blue) and somatostatin staining (green). Scale bar, 20 μm. (D) PP and somatostatin staining in islets was quantified by morphometry and islets were categorized into three groups according to the somatostatin staining (0, >0–5 and >5% of staining); the corresponding mean values for PP and somatostatin staining was calculated from islets (10–20) in each pancreas. Columns are the mean ± S.E.M. of 8–12 different pancreata. In islets with no somatostatin staining (0%), the PP staining was the highest, and in islets with the highest somatostatin staining (>5%), the PP staining was the lowest. *$P=0.001$ compared with 0% somatostatin staining; **$P<0.0001$ compared with the two other groups.

2 diabetic donors. Taken together, these results show that the islet cell organization is not affected by type 2 diabetes even if a consistent decrease in β cell mass has been demonstrated in these islets (Butler *et al*. 2003, Yoon *et al*. 2003).

When pancreas sections were labelled concomitantly for somatostatin and PP, we observed only few islets displaying both δ and PP cells (Fig. 5B). Of the islets displaying at least one δ or one PP cell, 23% comprised both δ and PP cells, whereas the majority (77%) were positive for δ or PP cells only (Fig. 5A and C). Then, somatostatin and PP staining in islets was quantified by computer-assisted morphometry and expressed as percentage of total islet area. Somatostatin staining was 3.8 ± 0.33% (mean ± S.E.M. of 132 islets) and PP staining was 2.7 ± 0.23% (mean ± S.E.M. of 132 islets). In each pancreas, islets were arbitrarily classified into three groups according to the somatostatin staining: 0%, 0 to 5% and more than 5%,

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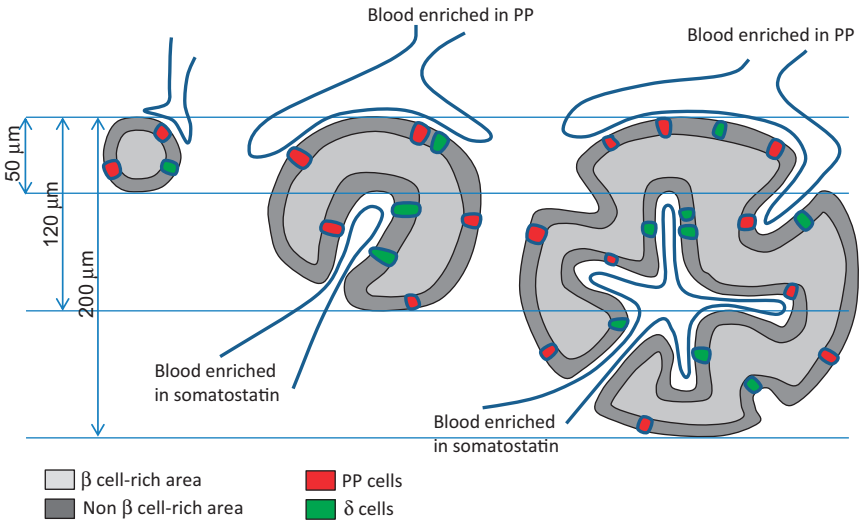

Figure 6
Model of a double vascularization in islets. Three islets with increasing size (50, 120 and 200 µm diameters) are depicted. PP cells are represented in red and δ cells in green. Blood vessels at the islet periphery and within intraislet vascular channels are shown in blue. Small islets (50 µm) are without intraislet vascular channel. In this model, blood circulating at the periphery of islet is enriched in PP, whereas blood circulating within intraislet vascular channels is enriched in somatostatin.

and the corresponding PP staining calculated (Fig. 5D). In islets with no somatostatin staining, the PP staining was the highest (11 ± 2.8%), and in islets with the highest somatostatin staining, the PP staining was the lowest (0.52 ± 0.32%) (Fig. 5D). Similar results were obtained with T2D islets (not shown). These results indicate that in islets, the amount of δ cells negatively correlates with the amount of PP cells.

Discussion

The main finding of this study is the asymmetric distributions of δ and PP cells between two different islet regions: the periphery and the intraislet vascular channels. Considering the trilaminar epithelial band model previously described (Bosco *et al.* 2010), one can conclude that one side of the epithelial band, bordered by invaginating vessels, is enriched in δ cells and the other side, bordered by peripheral vessels, is enriched in PP cells. The question arising from this observation pertains to the functional significance of this unique spatial organization of δ and PP cells. From a paracrine point of view, the consequence is that intercellular interactions are more limited between δ and PP cells than between the other cell types. The role of δ and PP cell interactions in islet physiology is not well understood, even if it has been shown that both δ and PP cells can inhibit one another (Bloom *et al.* 1980, Kim *et al.* 2014). With regard to their effects on insulin and glucagon release, both somatostatin and PP have been shown to have inhibitory effects (Lundquist *et al.* 1979, Bloom *et al.* 1980, Aragon *et al.* 2015). Somatostatin is a short living peptide with a half-life of < 1 min in the circulation, suggesting that its action is mediated principally through a local intraislet paracrine route. As both insulin and somatostatin secretions from β and δ cells, respectively, are increased by glucose, the resulting effect of somatostatin on β cells is to moderate the glucose-induced insulin secretion. The effect of somatostatin on α cells could be even more critical considering that the secretion of somatostatin by δ cells at high glucose could be required to inhibit glucagon secretion. In somatostatin knockout mice, the inhibition of glucagon secretion in response to glucose was absent (Hauge-Evans *et al.* 2009), indicating that δ cell somatostatin plays an important role in the inhibition of glucagon secretion in response to increases in plasma glucose and suggesting that glucose-induced somatostatin release is a major contributor to the inhibitory effects of glucose on islet α cells. Considering the preferential location of δ cells within the central part of the islets, it is tempting to suggest that all α or β cells within an islet are not similarly regulated by somatostatin. Both α and β cells have been shown to display different types of somatostatin receptors (SSTR) with variable affinities for the somatostatin secreted by δ cells (Mandarino *et al.* 1981, Francis *et al.* 1990, Kumar *et al.* 1999). It is therefore conceivable that islet cells could be heterogeneous for SSTR expressions and it would be interesting to investigate whether β or α cells lining intraislet vessels and contacting δ cells express distinct types of SSTR as compared to α or β cells at the islet periphery. PP has a most probable paracrine effect on α cells than on β cells. Indeed, one main PP receptor (PPYR1) was shown to be expressed on α cells and not on β cells. Furthermore, PP was shown to decrease glucagon secretion and to have no effect on insulin secretion (Aragon *et al.* 2015), suggesting that PP cells can mediate the inhibitory effect of glucose

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on glucagon secretion. Similarly to what proposed for δ cells, we can speculate that α cells close to PP cells, i.e. α cells at the periphery of islets, are more sensitive to PP than the other α cells. A functional significance of the specific δ and PP cell location in human islets can also be hypothesized taking into account the normal endocrine fate of islet hormones through vascularization. Blood vessels lining the islet periphery and those penetrating inside the islet might be from distinct branches and convey blood with different hormone compositions, i.e. enriched with either PP or somatostatin (a model is depicted in Fig. 6). As a consequence, parenchymal cells might be exposed to different hormone cocktails enriched with either somatostatin or PP, depending on the vascular branches. The secretory activity and the amylase content were shown to be different between peri-insular and other acini (Putzke & Said 1975, Aughsteen & Kataoka 1994), suggesting that hormones secreted by islets may affect the function of adjacent acinar cells. Considering the possible role of PP on pancreatic exocrine secretion (Putnam *et al.* 1989, Morisset 2008) and that hormone delivery to target cells may be mediated via the interstitial compartment, it makes sense that PP cells are mostly located at the periphery of islets.

Distributions of islet cells around the vascular channels and at the islet periphery were similar between the head and the tail of the pancreas. This result does not exclude more subtle and/or quantitative differences between islets from tail and the head of the pancreas. Indeed, analysis were designed here to study differential distributions of cells between the different islet areas, and it was beyond the scope of this study to analyse other qualitative or quantitative differences in islet cell distribution between different pancreas areas.

From a developmental point of view, it would be interesting to study how and when this differential distribution of δ and PP cells arises and whether it is related to differential expressions of transcription factors, and more specifically those that drive islet progenitors towards δ or PP cell differentiation. Notably, the expression of the *Arx* gene has been shown to be crucial in promoting islet progenitors towards the α and PP cell lineages (Collombat *et al.* 2007), as its inhibition induced by the *Pax* gene is required to form β and δ cells (Napolitano *et al.* 2015). It is totally unknown whether the location of δ and PP cells in islets is also dictated by differential expressions of these or other genes. Our analyses further show that no major islet architectural default is associated with type 2 diabetes. Thus, our results suggest that the dysfunction of insulin secretion in type 2 diabetes is not originated by a structural defect involving paracrine interactions between different islet cell types. Previous works have shown that anomalies in islet structure, consisting of intermingled α and δ cells with β cells, were detected in mouse models of obesity and type 2 diabetes (Starich *et al.* 1991, Kim *et al.* 2009). Similarly, in transgenic mice overexpressing hepatocyte nuclear factor 6, α, δ and PP cells were abnormally mixed with β cells (Gannon *et al.* 2000). It was suggested that islet cells established inappropriate intercellular interactions, resulting in perturbed islet cell function and contributing to diabetes in these animal models (Starich *et al.* 1991, Kim *et al.* 2009) (Gannon *et al.* 2000). Our results do not confirm these conclusions; however, additional studies should be conducted to understand whether some subtle defects in islet cell organization could be associated with type 2 diabetes in humans. With regard to type 1 diabetes, a clear description of human islet cell organization has not been reported yet; however, it is obvious that the severe destruction of β cells within type 1 diabetic islets results in a perturbed arrangement of the other islet cell types. Precise arrangement of cell-to-cell as well as cell-to-vasculature interactions in type 1 diabetic human islets deserves further detailed investigations.

In summary, these results indicate that δ and PP cells are found mostly in distinct islets and, when present within the same islets, are most often in separate compartments. Thus, these cell types have a propensity to be located away from one another. Further studies are needed to determine whether cell-to-cell or paracrine interactions between δ and PP cells are essential or not for an adequate control of islet hormone release. Finally, these results raise the possibility that the blood hormonal composition in pancreatic microvascularization might differ according to the origin of the islet blood vessels.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-15-0542.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

D B and C B researched and analysed the data and wrote the manuscript. T B contributed to interpretation of the data and revised the manuscript. G P, V L, E B, J M, M A A and E B researched and analysed the data. All authors have approved the final version of the paper.

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