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Heterogeneous distribution of plasma membrane glycoconjugates in pancreatic acinar cells

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Abstract

Flow-cytometric studies of lectin binding to individual acinar cells have been carried out in order to analyse the distribution of membrane glycoconjugates in cells from different areas of the pancreas: duodenal lobule (head) and splenic lobule (body and tail). The following fluoresceinated lectins were used: wheat germ agglutinin (WGA), *Tetragonolobus purpureus* agglutinin (TP) and concanavalin A (Con A), which specifically bind to *N*-acetyl D-glucosamine and sialic acid, L-fucose and D-mannose, respectively. In both pancreatic areas, two cell populations (R1 and R2) were identified according to the forward scatter (size). On the basis of their glycoconjugate pattern, R1 cells displayed higher density of WGA and TP receptors than R2 cells throughout the pancreas. Although no difference in size was found between the cells from duodenal and splenic lobules, *N*-acetyl D-glucosamine and/or sialic acid and L-fucose residues were more abundant in plasma membrane cell glycoconjugates from the duodenal lobule. The results provide evidence for biochemical heterogeneity among individual pancreatic cells according to the distribution of plasma membrane glycoconjugates.

Keywords: Lectin; Pancreatic cell; Plasma membrane glycoconjugate; (Rat)

1. Introduction

The pancreas is an elongated organ with regions arising from different embryologic origin [1] and whose visceral and vascular relations are also different. The thousands of individual acinar cells that form the exocrine pancreas appear relatively homogeneous in either light or electron microscopy. However, there is an increasing number of reports which support the point of view that the exocrine pancreas would be considered as a heterogeneous organ. This notion is based on increasing physiological data which includes selective pancreatic responses to different stimuli [2–9]. It is well established that the functional state of the pancreas greatly depends on the interaction of neurotransmitters and hormones with highly glycosylated cell membrane receptors [10–13]. Despite this, little is known about whether a heterogeneity can be established in the distribution of cell surface saccharide within different regions of the gland.

Lectins are sugar-binding proteins which have proved to be useful tools for the detection of glycoconjugates in pancreatic tissue sections [14,15], pancreatic cells [16,17] and isolated pancreatic zymogen granules [18–20].

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The aim of this study was to analyse by flow cytometry the lectin binding to individual pancreatic cells isolated from the two gross regions of the rat pancreas (duodenal and splenic lobules) which embryologically originate from ventral and dorsal buds, in order to investigate whether a regional heterogeneity in the distribution of plasma membrane glycoconjugates can be detected.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin, collagenase type VII, soybean trypsin inhibitor, amino acid admixture, FITC-conjugated lectins (wheat germ agglutinin (WGA–FITC), *Tetragonolobus purpureus* (TP–FITC) and concanavalin A (Con A–FITC)), *N*-acetyl D-glucosamine, sialic acids, L-fucose, D-mannose and D-glucose were purchased from Sigma Chemical Co. (Madrid, Spain). Fix cell kit was supplied by Caltag (Burlingame, CA). The rest of the reagents were obtained from Merck (Darmstadt, Germany).

2.2. Isolation of pancreatic cells

Male Wistar rats weighing about 250 g were used. After overnight fasting the rats were anaesthetised with sodium pentobarbital (3 mg/100 g body weight, intraperitoneally). The bile duct was ligated at its exit from the liver and the main pancreatic duct was cannulated at its exit into duodenum to perfuse 5 ml of 25 mM Hepes solution (pH 7.4) containing collagenase (40 U/ml), 0.1 mg/ml soybean trypsin inhibitor, 100 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 14 mM D-glucose, 2 mM glutamine, 2% (w/v) bovine serum albumin and 2% (w/v) amino acid mixture. The solution was pre-balanced with 95% O_2 and 5% CO_2 and all posterior incubations were performed with this gas phase. The pancreas was removed and minced at the superior mesenteric vessels in order to separate the anterior part closest to the duodenum (head) from the remainder that reaches the spleen (body and tail). These two areas will be referred to as duodenal lobule and splenic lobule, respectively. Each pancreatic region was digested at 37°C in a shaking bath (200 cycles/min) during 20 min and washed with fresh collagenase solution every 5 min. Following gentle pipetting through tips of decreasing diameter (3–1 mm), cells were filtered through a double layer of muslin gauze and centrifuged at 30 g for 30 s at 4°C. The supernatant was collected and centrifuged at $500\times g$, 5 min, 4°C. The supernatant was now removed and the pellet resuspended in Hepes buffer without collagenase and centrifuged again ($500\times g$, 5 min, 4°C). The study protocol was approved by the Ethic Committee of the Salamanca University.

2.3. Staining of cells with FITC-lectins

One hundred μl of a suspension of pancreatic cells (10^6/ml) were fixed by incubation with 100 μl of Fix for 15 min. Then 0.1 M saline phosphate buffer (pH 7.4) was added and the cells were washed by centrifugation ($2\times 500g$, 5 min). The pellet was resuspended in saline phosphate buffer and incubated for 15 min in the dark with one of the following fluoresceinated lectins: WGA–FITC, TP–FITC or Con A–FITC, at saturating concentrations (1.5 μg/μl). Unbound FITC-lectin was removed by centrifugation twice in saline phosphate buffer at $500\times g$ for 5 min. The specificity of the binding was assessed by using the same concentration of lectin as a control but in the presence of 0.2 M *N*-acetyl D-glucosamine and sialic acid for WGA–FITC, L-fucose for TP–FITC and D-mannose and D-glucose for Con A–FITC.

2.4. Flow cytometry measurements

A Facscan flow cytometer (FACSCalibur, Becton/Dickinson) equipped with an argon ion laser tuned at 488 nm and 15 mW was used. During the whole measurement process the pancreatic cells remained resuspended in 0.1 M saline phosphate buffer (pH 7.4) which was surrounded by the sheath fluid (Facsflow, Becton/Dickinson) so that no admixture could occur between the two fluids, in accordance with the principle of the Facscan flow cytometer [21]. Calibration of the instrument was performed on a daily basis using Calibrite beads (Becton/Dickinson). Fine adjustments were performed using unstained cells and fluorescence compensation was established by measuring FITC-labelled cells. The Lysis II soft-

ware programme was used for data acquisition. In each experiment, the mean value per individual cell was obtained from the analysis of at least 10 000 cells and it was calculated using Cell Quest software.

2.5. Statistical analysis

Results were expressed as means ± S.E.M. A paired Student's *t*-test was applied in order to establish whether the differences between the cells from two duodenal and splenic lobules were statistically significant. This statistical test was also used for the comparison between the two subsets of cells differentiated by flow cytometry. *P* values lower than 0.05 were considered to be statistically significant.

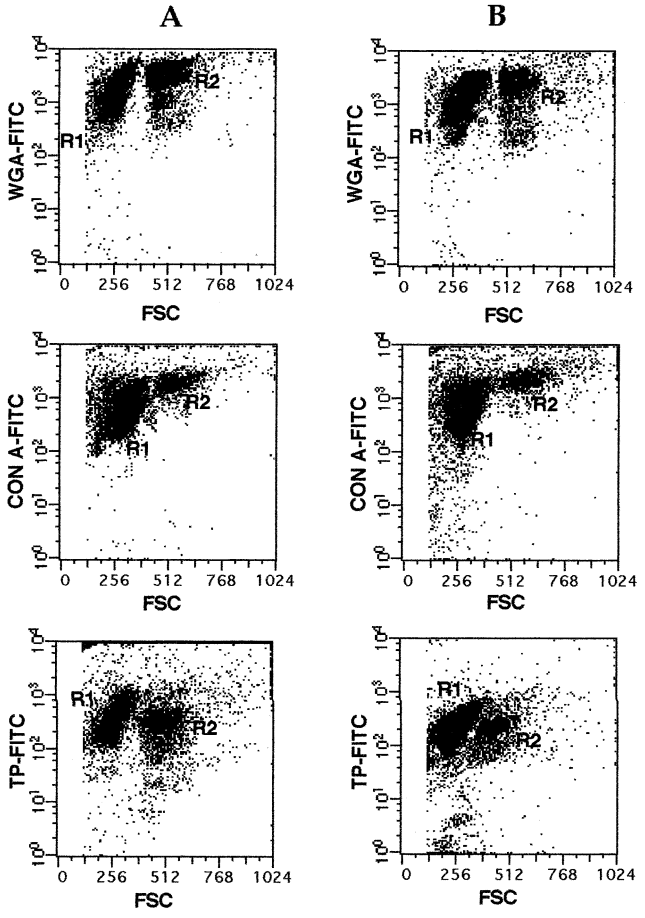

Fig. 1. Flow-cytometric distribution of pancreatic cells from duodenal lobule (column A) and splenic lobule (column B) according to forward scatter (FSC) and fluoresceinated lectins (FSC/WGA–FITC, FSC/Con A–FITC, FSC/TP–FITC). Each dot plot is a representative example of one experiment.

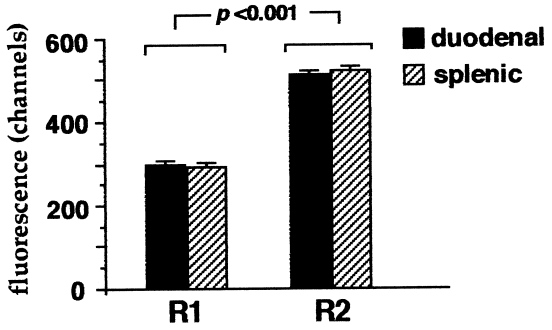

Fig. 2. Forward scatter of pancreatic cells from duodenal ($n = 18$) and splenic ($n = 18$) lobules. n = number of experiments. Values are mean channels ± S.E.M. (arbitrary units scaled from 0 to 10 000). Paired Student's *t*-test showed statistical significant differences between R1 and R2 cells.

3. Results

Fig. 1 is a dot plot set that shows a representative example of the flow-cytometric distribution of pancreatic cells from duodenal and splenic lobules according to FSC (forward or low-angle scatter) and the lectin labelling (WGA–FITC, Con A–FITC and TP–FITC). Two populations of cells are clearly differentiated in both areas on the basis of FSC which are referred to as R1 and R2. From the distributions a high degree of heterogeneity according to the lectin binding can be observed.

Fig. 2 shows the mean FSC values of R1 and R2 cells. No significant difference was observed between the two pancreatic areas, but in both regions, R2 cells displayed significantly ($P < 0.001$) higher FSC values than R1 cells.

The measurements of lectin binding revealed significantly higher ($P < 0.001$) WGA and Con A labelling in R2 than in R1 cells in both the duodenal and splenic lobules (Table 1). However, the analysis of lectin binding/FSC ratio (Fig. 3) revealed that in the two pancreatic areas the values for WGA and TP lectins were significantly higher in R1 cells than in R2, while no significant difference between the two cell populations was found for ConA/FSC ratio.

As shown in Fig. 4, differences between cells from duodenal and splenic lobules were detected according to WGA and TP binding. R1 and R2 cells from the duodenal lobule showed significantly higher WGA ($P < 0.05$) labelling than cells from the splenic lobule. R1 cells from the duodenal lobule displayed significantly ($P < 0.01$) higher TP labelling than in the

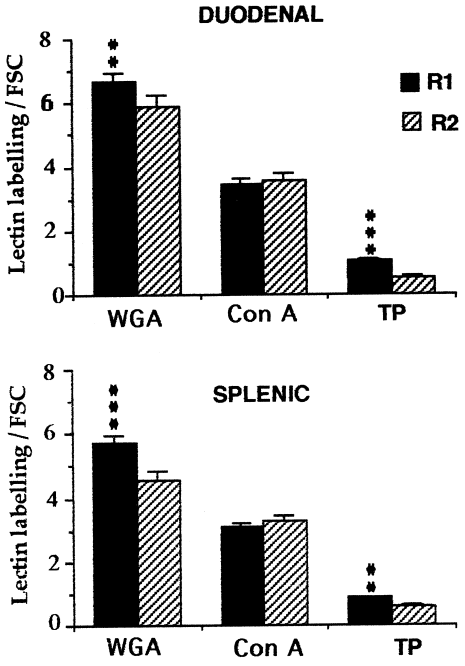

Fig. 3. Lectin binding (WGA, Con A and TP): forward scatter (FSC) ratio in R1 and R2 cells from duodenal and splenic lobules. Number of experiments: 5–8. Values are means ± S.E.M. Paired Student's *t*-test showed statistical significant differences between R1 and R2 cells (**$P < 0.01$, ***$P < 0.001$).

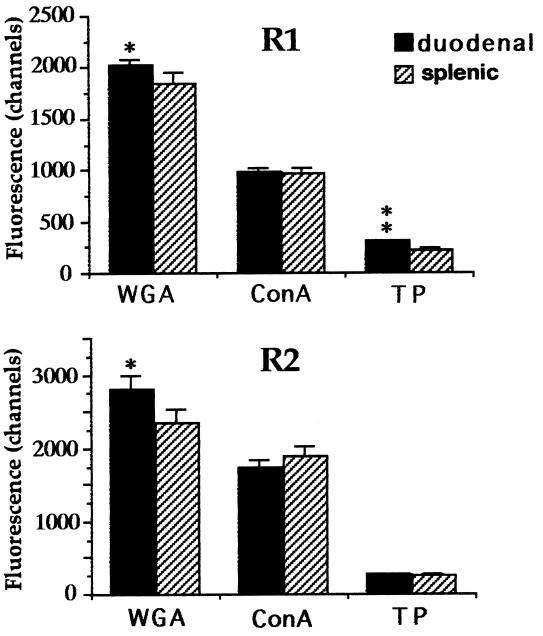

Fig. 4. WGA–FITC, Con A–FITC and TP–FITC binding to individual R1 and R2 cells from duodenal and splenic lobules. Number of experiments: 5–8. Values are mean channels ± S.E.M. (arbitrary units scaled from 0 to 10 000). Paired Student's *t*-test showed statistical significant differences between cells from duodenal and splenic lobes (*$P < 0.05$, **$P < 0.01$).

splenic lobule. No significant difference was found between the two pancreatic regions for Con A binding in either R1 or R2 cells.

4. Discussion

In humans the pancreas is a compact gland in which the terms of head, body and tail are used to designate regions of the organ from proximal to distal. In rodents the shape of the pancreas is rather less well defined and the area referred to as the duodenal lobule would represent the head while the splenic lobule would correspond to the rest (body and tail).

Morphometric analysis carried out by Bendayan [22] showed that the size of acinar cells is not uniform throughout the pancreas. In agreement with Bendayan' reports we have recently described by flow-cytometric assays [17] the existence of two populations of cells in the whole pancreas of the rat according to the forward scatter (FSC). FSC is a parameter related with the cross-sectional area of a particle measured by flow cytometry [23] and it reflects the cell size. The results obtained in cells isolated from duodenal and splenic pancreatic lobules

Table 1
Lectin–FITC labelling to R1 and R2 cells from pancreatic duodenal and splenic lobules

	Duodenal			Splenic		
	WGA	Con A	TP	WGA	Con A	TP
R1	2031 ± 55	988 ± 41	305 ± 13	1843 ± 101	962 ± 61	222 ± 9
R2	2953 ± 146***	1863 ± 113***	263 ± 26*	2460 ± 171***	1929 ± 90***	265 ± 21

Values are mean channels (arbitrary units scaled from 0 to 10 000) ± S.E.M. Paired Student's *t*-test revealed significant differences between R1 and R2 cells (*$P < 0.05$, ***$P < 0.001$).

show that neither cell population (R1 or R2) are restricted to particular areas of the gland but appear spread throughout organ.

Glycohistochemical studies carried out on pancreatic tissue sections and isolated acinar cells [14–16] have reported a strong binding of wheat germ agglutinin (WGA), *Ulex europeaus* agglutinin and concanavalin A (Con A) to the plasma membrane of pancreatic cells. Accordingly, we have chosen lectins which specifically bind the same sugar residues (WGA for *N*-acetyl D-glucosamine and sialic acid, *Tetragonolobus purpureus* agglutinin (TP) for L-fucose and Con A for D-mannose) which are located at different levels in the membrane glycoconjugates.

Analysis by flow cytometry have allowed for the first time quantitative comparisons of lectin-ligands on the pancreatic cell surfaces in order to check individual differences. R1 and R2 cells showed different plasma membrane glycoconjugate composition. As was observed in the whole pancreas [17], R2 cells from both duodenal and splenic lobules showed higher WGA and ConA labelling than R1 cells. However, according to the FSC values, the cells belonging to the R2 population are larger than R1 cells, so that the amount of lectin bound to their membrane could be influenced by the cell size. Therefore, it seems appropriate to consider the lectin/FSC ratio in the comparative analysis between R1 and R2 cells. Accordingly, in both pancreatic areas the density of receptors for WGA and TP lectins is higher in R1 cells, while no difference was found for Con A between the two cell populations. These results could be related to the degree of maturity of the cell, since D-mannose, which specifically binds to Con A, is located at early glycosylation steps, as part of a pentasaccharidic core where different saccharidic terminals are added at later steps [24]. In contrast, L-fucose, a carbohydrate found in abnormally high concentrations in pancreatic cells, is more abundant at terminal positions in the oligosaccharide chain [25,26] as well as *N*-acetyl D-glucosamine and a high amount of sialic acid [24]. As regards the expression of blood group antigen, heterogeneity in the membrane of human acinar cells was reported by Uchida et al. [27] who showed different acinar types spread widely throughout the gland which did not segregate into either gross pancreatic regions such as head and tail, or into centroacinar, ductular or peri- and teleinsular areas. The present study does not allow us to assess whether glycoproteins and/or glycolipids contribute to the differences observed in lectin binding. It is likely that they are both involved, but to a greater extent probably glycoproteins, since plasma membrane of acinar cells is provided with a high number of receptors for hormones and neurotransmitters which have demonstrated to be glycoproteins [10–13]. In any case, differences in the glycan structure are the most interesting from a functional point of view, since both hormones and neurotransmitters act on acinar cells by binding to the oligosaccharide portion of glycoconjugate.

On the other hand, no difference was found in the FSC values of two cell populations between duodenal and splenic lobules. Therefore, the capability of lectin binding to plasma membrane glycoconjugates in both pancreatic areas is not dependent on size. Interestingly, cells isolated from the duodenal lobule of the pancreas showed higher WGA and TP labelling. It has been reported that the pancreatic receptor for the gastrointestinal hormone cholecystokinin (CCK) is a glycosylated protein with high content in *N*-acetyl glucosamine, sialic acid and L-fucose residues [28–30], sugars to which WGA and TP lectins bind. Moreover, flow-cytometric studies carried out by Jonas et al. [16] showed two populations of cells in the rat pancreas according to CCK–FITC labelling which, regarding our results, could be preferentially located in different areas of the pancreas. All these data would suggest that cells belonging to the duodenal lobule would be more sensitive to CCK action. Two functionally different regions in the pancreas could be related to the embryology and development of the gland. In fact, the pancreas has its origin as two buds developing on the dorsal and the ventral side of the duodenum which fuse together to form a single organ [1].

Taking into account that CCK acts as an important trophic factor on the pancreas [31] and that it is also involved in the regulation of the exocrine function of the gland [32], the differences observed between cells from distinct pancreatic regions could be of interest in order to understand the different capacity for regenerating the pancreas in different experimental situations which lead to progressive destruction of the gland [33,34].

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