Rapid Communication

Presence of extrapancreatic islets of Langerhans in the duodenal wall of the rat

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Summary. In the present study we report the existence of typical islets of Langerhans in the duodenal wall of the rat. The nature of the cells and of their corresponding secretory granules were assessed by morpho-cytochemical techniques. The insulin cells formed the core of the islets while the glucagon, the somatostatin and the pancreatic polypeptide cells were located at the periphery. These islets were located in the connective tissue between the duodenal crypts and the

muscle layer, neighbouring the last portion of the bile duct. The morphological features of these endocrine cells indicate the presence of high secretory activities which may play an important role in the maintenance of glucose homeostasis, particularly during absorption of nutrients.

Key words: Islets of Langerhans, duodenal wall, immunocytochemistry, rat.

In 1869 Paul Langerhans described the presence of particular cellular clusters, the islets of Langerhans, in the pancreatic tissue. Since then, and in spite of numerous studies and major advances in the understanding of the morphological and functional properties of the islets of Langerhans [1], the presence of islets has been restricted to the pancreatic gland. In the present study, morpho-cytochemical investigations performed on the duodenal wall of normal adult rats have demonstrated the presence of islets of Langerhans displaying the four different pancreatic endocrine cells in their classic arrangement. The role of these extrapancreatic islets in the maintenance of glucose homeostasis remains to be elucidated although they must be involved in the rapid endocrine response to glucose absorption during digestion.

Materials and methods

Duodenal and pancreatic tissues from six male Sprague Dawley rats (~250 g body weight) were fixed in Bouin's fixative or in 4% paraformaldehyde-1% glutaraldehyde, 0.1 mol/l phosphate buffer and processed for light (paraffin) and electron microscopy (Epon), respectively. The duodenal tissue was sampled at different levels, particularly those contiguous to the last portion of the bile duct. For light microscopic immunohistochemistry, the avidin-biotin peroxidase technique [2] was applied in combination with four specific antibodies: a guinea-pig anti-insulin (ICN Immunobiologicals, Lisley, Ill., USA) and rabbits anti-glucagon, anti-somatostatin (Instar Corp., Stillwater, Minn., USA) and anti-pancreatic polypeptide (Dako Corp., Carpinteria, Calif, USA) antibodies. For electron microscopic immunocytochemistry, the protein A-gold technique [3] was applied in combination with the same antibodies.

Results

Examination of duodenal tissue sections after the peroxidase staining with the four different antibodies, revealed positive cells in the connective tissue of the duodenal mucosa and submucosa (Figs. 1 a–d). The insulin (Fig. 1 b) and glucagon (Fig. 1 d) cells were arranged in clusters which resembled the pancreatic islets. These extrapancreatic islets were located between the duodenal crypts and the muscle layer, but were restricted to the area of the duodenum traversed by the last portion of the bile duct before its opening into the intestinal lumen. The islets were not located in the wall of the duct, but rather close to the duodenal crypts. In several instances, a small accessory pancreatic duct which directly connects the duodenal lumen, was found in the vicinity (Fig. 1) and isolated endocrine cells were occasionally present among the epithelial cells (Fig. 1 c). In all cases, the islets were clearly surrounded by connective tissue and showed no direct contact with the epithelial cells of the ducts or of the crypts. No acinar tissue was present in the surroundings. When serial sections were performed and stained for the different pancreatic hormones, the classic organization described for the pancreatic islets [1] was found in these extrapancreatic ones. Indeed, the majority of the insulin-positive cells constitute the core of these islets while the glucagon and some somatostatin- and pancreatic polypeptide-secreting cells were located at the periphery displaying thin cytoplasmic processes (Figs. 1 a, 1 c) that encircle in part the other cells. These observations were confirmed by electron micro-

Fig. 1a-d. Light micrographs of serial sections of the rat duodenal wall stained with the anti-pancreatic polypeptide (a), anti-insulin (b), anti-somatostatin (c), and anti-glucagon (d) antibodies. The insulin (b) and glucagon (d) cells form two large islets. The pancreatic polypeptide (a) and somatostatin (c) cells display only thin cytoplasmic processes in the same islets. One somatostatin cell (arrowhead) is also present in the epithelium of the accessory pancreatic duct (D). DC, duodenal crypt. $\times 750$

Fig. 2. Electron micrograph of an islet of Langerhans present in the duodenal wall of the rat. The islet is located in the connective tissue,

between the duodenal crypts (DC) and the muscle layers (M). Glucagon (A) and insulin (B) cells are easily identifiable in this section of the islet. Numerous capillaries (Ca) are present around and inside the islet. $\times 3000$

Fig. 3a-d. Immunogold labelling for various peptides in islet cells using the anti-pancreatic polypeptide (a), anti-insulin (b), anti-somatostatin (c), and anti-glucagon (d) antibodies. In each case the gold particles are present on the core of the corresponding secretory granules. a and d $\times 38000$, b and c $\times 30000$

scopy (Fig. 2) which in addition revealed the characteristics of each type of cell. In particular, the morphological features of the secretory granules which identify each cell type concurred with those well established in the corresponding pancreatic endocrine cells. In addition to the presence of numerous secretory granules, the endocrine cells displayed well-developed rough endoplasmic reticulum and Golgi apparatus. Several granules were located at the periphery of the cells closely related to the plasma membrane. The hormonal content of each granule was confirmed by performing the protein A-immunogold technique with the appropriate antibody. Indeed, the labelling by gold particles revealing the corresponding antigens, was present over the core of the appropriate granules (Fig. 3), as well as over the endoplasmic reticulum and Golgi. Numerous capillaries were found in the connective tissue closely related to the islet cells with occasional penetration through the islet itself. Furthermore, bundles of nerve fibres originating from neighbouring submucosal ganglia, reached the peripheral islet cells. The presence of such extrapancreatic islets was registered in four of the six animals investigated.

Discussion

In the present report we describe the presence of typical islets of Langerhans in the duodenal wall. These islets were located in the region traversed by the last portion of the bile duct. Besides being located extrapancreatically and away from the acinar parenchyma, the morphological characteristics of these islets and of their cellular components were found, by all means, to be identical to those displayed by the pancreatic islets. Furthermore, the intracellular compartments and secretory machinery of each cell type were equally identical to those well established in pancreatic islet cells. The fact that all the cell types display well-developed rough endoplasmic reticulum, large Golgi apparatus and numerous secretory granules, some of which were located close to the plasma membrane, speaks in favour of a high secretory activity in these endocrine cells. The relevance of their secretion on blood glucose homeostasis, remains however to be evaluated. Because of their topographical location, these islets might be exposed to high interstitial glucose levels, particularly at time of digestion and absorption. Their secretion may thus counteract rapid and transient, but local, hyperglycaemia due to massive glucose absorption. It is of interest to underline the existence of a group of disorders in which hypoglycaemia occurs in post-fed states [4]. The causes of this postprandial reactive hypoglycaemia remain undetermined and controversial. That this idiopathic reactive hypoglycaemia is related to the functional activity of the duodenal islets, could be considered.

Another role that pancreatic hormones may play in the intestinal wall corresponds to a trophic paracrine action on surrounding tissues. Indeed, pancreatic hormones have been shown to have a direct and definite influence on the pancreatic exocrine parenchyma [5], through the presence of specific hormone receptors at the level of the acinar cell plasma membranes. Similarly, insulin receptors have been described in the baso-lateral plasma membrane of enterocytes [6, 7], and insulin has been shown to have a

trophic effect on duodenal epithelial cells [8, 9]. Thus, the presence of functional extrapancreatic islets in the intestinal mucosa, may result in a direct paracrine influence of islet hormones on duodenal epithelium.

Concerning the origin of these islets, it has been clearly established that along the phylogeny, isolated endocrine cells secreting pancreatic hormones were first located in the gut mucosa of invertebrates [10]. In cyclostomes the first separated islet organ, embedded in gut-connective tissue is found in the distal part of the bile duct protruding into the gut [10]. In highly developed teleost, on the other hand, the Brockmann bodies remain related to the bile duct although the endocrine tissue is already well detached from the gastrointestinal tract. Thus, the presence of islet cells in the duodenal wall of mammals, while not being open to the gut lumen but closely related to the bile duct, appears to be an evolutionary remnant from the phylogenetic evolution which could still play an important role in the maintenance of glucose homeostasis.

Finally, in searching for adequate sites for transplanting pancreatic tissue, the normal occurrence of islets of Langerhans in the duodenal mucosa of mammals, indicates that this intestinal site may in fact represent a particularly favourable location for islet transplantation.

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References

- Orci L (1985) The insulin factory: a tour of the plant surroundings and a visit to the assembly line. *Diabetologia* 28: 528–546
- Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 29: 577–580
- Bendayan M (1984) Protein A-gold electron microscopic immunocytochemistry: Methods, applications and limitations. *J Elect Microsc Tech* 1: 243–270
- Hofeldt FD (1989) Reactive hypoglycaemia. In: Field JB (ed) *Endocrinology and metabolism clinics of North America*, Vol 18/1. Saunders, Philadelphia, pp 185–201
- Bendayan M (1990) Anatomical basis of islet-acinar interaction in the pancreas. *Reg Pept Let* 11: 1–7
- Bergeron JJM, Rachubinski R, Searle N, Borts D, Sikstrom R, Posner BI (1980) Polypeptide hormone receptors, in vivo: Demonstration of insulin binding to adrenal gland and gastrointestinal epithelium by quantitative radioautography. *J Histochem Cytochem* 28: 824–835
- Gallo-Payet N, Hugon JS (1984) Insulin receptors in isolated adult mouse intestinal cells: Studies in vivo and in organ culture. *Endocrinology* 114: 1885–1892
- Ménard D, Malo C, Calvert R (1981) Insulin accelerates the development of intestinal brush border hydrolytic activities of suckling mice. *Dev Biol* 85: 150–155
- Kellett GL, Jamal A, Robertson JP, Wollen N (1984) The acute regulation of glucose absorption, transport and metabolism in rat small intestine by insulin in vivo. *Biochem J* 219: 1027–1035
- Falkmer S (1979) Comparative morphology of pancreatic islets in animals. In: Wolk BW, Arquilla ER (eds). *The Diabetic Pancreas*. Plenum Press, New York pp 17–52

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