Immunohistochemical Analysis of the Endocrine Cells in the Pancreatic Islets of Cattle

By

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Summary: The distribution of endocrine cells and the areas of islets in the bovine pancreas were investigated by immunohistochemical methods. The islets in the A-region, which consisted of the left lobe, the ventral portion of the body, and the ventral and distal portions of the right lobe, contained a central core of insulin-containing (B-) cells surrounded by glucagon-containing (A-) cells, a few somatostatin-containing (D-) cells and sporadic pancreatic polypeptide-containing (PP-) cells. The islets in the B-region, which consisted of the uncinate process, and the dorsal and proximal portions of the body and the right lobe, contained a central mass of B-cells, and peripheral cells which were predominantly PP-cells with a few D-cells, but no A-cells. The areas of islets in the B-region were small as compared with those in the A-region. From these findings it is suggested that A- and PP-cells have a complementary relationship to one another. In view of this hypothesis, two types of islet, A-cell-rich and PP-cell-rich islets, were identified. The A-region contained A-cell-rich islets derived from the dorsal pancreatic primordium, while the B-region contained PP-cell-rich islets that originated from the ventral primordium. In the bovine pancreas, the areas containing PP-cell-rich islets are greater than those in previously examined species.

The pancreas originates embryologically from the dorsal and ventral pancreatic primordia, which fuse and develop into the pancreas (O’Rahilly and Müller, 1978; Noden and De Lahunta, 1985; Sadler, 1985). In the human, the caudal part of the head and the uncinate process are derived from the ventral pancreatic primordium, while the cephalic part of the head, the body and the tail are derived from the dorsal pancreatic primordium (O’Rahilly and Müller, 1978; Sadler, 1985). However, there are no reports about the development of the bovine pancreas to our knowledge. It is generally assumed that four types of endocrine cell, namely, insulin-containing (B-), glucagon-containing (A-), somatostatin-containing (D-) and pancreatic polypeptide-containing (PP-) cells are present throughout all the islets in the pancreas of most mammalian species, with the B-cells being the most numerous (Fujita and Fujita, 1992). Previous immunohistochemical studies have clearly revealed the varied distribution of two types of islet on the basis of the relative sizes of populations of A-cells and PP-cells (Orci et al., 1976; Gersell et al., 1978; Paulin and Dubois, 1978; Baetens et al., 1979; Malaisse-Lagae et al., 1979; Rahier et al., 1979, 1981; Fururoka et al., 1989; Furuzawa et al., 1992; Takeuchi et al., 1994).

This paper describes the results of a systematic immunohistochemical study of the bovine pancreas that was designed to identify the two types of islet and to clarify the existence of two distinct populations of islets that differ in terms of cellular contents and topographical distribution.

Materials and Methods

The pancreata from seven adult female Holstein Friesian cows, obtained from a slaughterhouse, were used. Small portions of pancreatic tissue from the regions shown in Figure 1 were sampled. Tissue blocks were fixed for two days in Bouin’s solution at room temperature, and then they were processed routinely for embedding in paraffin. Sections were cut at 5 μm.

Sections were stained immunohistochemically by the avidin-biotin-peroxidase complex (ABC) method (Hsu et al., 1981). The antisera used in this study were rabbit antiserum against synthetic porcine glucagon (diluted 1:5,000; Amersham, Buckinghamshire, U.K.),...
guinea pig antiserum against bovine/porcine insulin (1:10,000; Incstar, Stillwater, Minnesota, U.S.A.), rabbit antiserum against synthetic human somatostatin-14 (1:4,000; Cambridge Research Biochemicals, Northwich, Cheshire, U.K.) and rabbit antiserum against human pancreatic polypeptide (1:10,000; Chemicon, Temecula, California, U.S.A.). After immunostaining, sections were counterstained with Mayer’s hematoxylin. To assess the specificity of the immunohistochemical staining, normal goat serum was substituted for each specific primary antiserum. Controls that has been prepared by replacing each primary antiserum with normal goat serum gave negative all the immunohistochemical reactions in all cases.

Determination of the cellular composition was based on the ratios of areas each type of cell in an islet to the area of islet (Table 1) (Furuzawa et al., 1992). The areas and constituent cells of islets were traced on sections, showed in digitized form in a personal computer (PC-9801 DA 32 Bit; NEC, Tokyo), and processed by use of appropriate software (OZ; OLYMPUS, Tokyo).

Results

The pancreas of each cow consisted of a large right lobe (lobus dexter), a smaller left lobe (lobus sinister) and the body (corpus) at the junction of the right and left lobes (Habel, 1975; Schummer et al., 1979). In addition to these three regions, there was an uncinate process that projected laterally from the body (Fig. 1). This small process belongs to the right lobe according to Nomina Anatomica Veterinaria Japonica (N.A.V.J.) (Japanese Association of Veterinary Anatomists, 1987). The right lobe extended caudally along the descending duodenum. Only a single accessory pancreatic duct (of the dorsal pancreatic primordium) emerged from the caudal end of the right lobe, and it opened into the duodenum about 20 cm caudal to the common bile duct (Fig. 1; APD). The cranial portion of the right lobe covered most of the ventral portion of the body, as well as the ventral portion of the right lobe about 10 cm caudal to the region along the descending duodenum (Fig. 1; hatched area).

Four kinds of immunoreactive cell, namely, A-, B-, D-, and PP-cells, were clearly visualized in the islets.

![Fig. 1. A diagram showing the various regions of the bovine pancreas (ventral view).](image-url)

L, Left lobe (white area); UP, uncinate process (black area); B, body (black and hatched area); R, right lobe (white, black and hatched area); a, ventral portion of body; b, proximal portion of body; c, dorsal portion of body; d, distal portion of right lobe; e, ventral portion of right lobe; f, proximal portion of right lobe; g, dorsal portion of right lobe; APD, accessory pancreatic duct. Cross-sectioned profiles are shown as arrows.
They also were detected in the exocrine parenchyma. The distribution of these endocrine cells in the islets of the four regions of the pancreas is shown in Table 1.

For convenience, we refer here to the left lobe, the ventral portion of the body, and the ventral and distal portions of the right lobe as the A-region, and to the uncinate process, and the dorsal and proximal portions of the body and the right lobe as the B-region.

A-cells were present in clusters within islets or were disseminated around clusters of B-cells in the A-region (Fig. 2a), and they accounted for 15 to 17% of the cell population (Table 1). A-cells were never found in the B-region (Fig. 1; black area, Fig. 3a).

B-cells were consistently observed as a core within islets of all portions and accounted for a majority (66 to 78%) of cells (Figs. 2b, 3b, Table 1).

D-cells accounted for only 3 to 6% of the cell population and were distributed between regions of A- and B-cells or among the B-cells in every islet in all regions (Figs. 2c, 3c, Table 1).

A few PP-cells were found in the islets of the A-region (Fig. 2d). PP-cells were mainly seen at the periphery of islets and they were occasionally distributed at random within islets in all regions (Figs. 3d, 5b). In the A- and B-regions, PP-cells were often found in the exocrine parenchyma (Fig. 4). The relative size of the population of PP-cells in islets in the B-region was much greater than that in the A-region (Table 1). No islets consisting of PP-cells exclusively were found.

We classified the pancreatic islets in the cows into two types on the basis of their cellular composition. The first type of islet, the PP-cell-rich islet, was observed in the B-region, and it was composed of B-, PP- and D-cells, with no A-cells (Fig. 3a—d, Table 1). The second type of islet, the A-cell-rich islet, was located in the A-region, and it was composed of B-, A-, D-, and PP-cells. The relative level of PP-cells in this type of islet was very low, ranging from 0.9 to 1.2% (Table 1). No significant differences were seen in the cellular composition of A-cell-rich islets in the A-region excluding B-region (Table 1).

There were boundaries between the dorsal portions of the body and the right lobe which included the PP-cell-rich islets, and between the ventral regions of the body and the right lobe that contained A-cell-rich islets (Fig. 5a, b). These boundaries could be recognized microscopically from the wide pattern of spread distribution of PP-cells (Fig. 5b).

Each pancreatic portion was composed of a great number of lobules that were separated from one another by a small amount of connective tissue. The islets were mainly dumbbell-shape, and the connective tissue extended into the islets. The mean area of islets in the B-region was significantly smaller than that in the A-region (Table 1).

Discussion

The bovine pancreas is divided into the left lobe, the right lobe and the body in standard textbooks (Habel, 1975; Schummer et al., 1979). Although the term “uncinate process” is used in the human pancreas, it was not been used in the bovine pancreas (Habel, 1975; O’Rahilly and Müller, 1978; Schummer et al., 1979; Sadler, 1985). However, in this study, we use this term to refer to a projection from the body of the bovine pancreas.

In the present study, almost all the pancreatic islets in the bovine were found to be composed of A-, B-, D-, and PP-cells, as already reported in many species of mammals including the human (Larsson et al., 1976; Fujita and Fujita, 1992). The relative proportions of A-, B-, D-, and PP-cells were determined in sequentially immunostained serial sections. The mean value for each
type of cell showed a tendency similar in terms of the relative proportion of islet cells to that in other mammals, decreasing in the order B-, A-, D-, and PP-cells, with the exception of PP-cell-rich islets.

In the islet of the rat (Orci et al., 1976; Baetens et al., 1979), human (Gersell et al., 1978; Paulin and Dubois, 1978; Malaisse-Lagae et al., 1979; Rahier et al., 1979, 1981), dog (Gersell et al., 1978), cat (Furuzawa et al., 1992) and gerbil (Takeuchi et al., 1994), B-cells formed the central core, and A-, D- and PP-cells were located at the periphery of the islets. In the cows studied here, the pancreatic islets were made up of compact masses of B-cells with a thin rim or a scattering of A-, D- and PP-cells. The converse pattern of distribution of A- and B-cells has been described in the horse (Furuoka et al., 1989).

The different distribution of the various endocrine cells in islets in each portion of the pancreas was confirmed by morphometric analyses of the four types of cell. The differences in population are due to a complementary relationship between A- and PP-cells.

In the present study, preferential location of PP-cells was observed in the B-region in the bovine pancreas (Bonner-Weir and Like, 1980; Nakajima et al., 1988), and these portions were completely devoid of A-cells. By contrast, the uncinate process in the human pancreas has a few A-cells (Gersell et al., 1978; Paulin and Dubois, 1978; Malaisse-Lagae et al., 1979; Rahier et al., 1979, 1981). The duodenal portion of the cat pancreas does, however, lack A-cells (Furuzawa et al., 1992).

In the present study, the size of the D-cell population was significantly lower in the B-region, clearly resembling more closely the A-cell population and being the highest in the ventral portion of the right lobe. Similar observations have been reported in the pancreas of the dog (Gersell et al., 1978) and the cat (Furuzawa et al., 1992).

The different arrangements of A-cells and PP-cells suggested that there are two types of islet, A-cell-rich islets and PP-cell-rich islets, in the bovine pancreas. This observation corresponds to results reported in the rat (Orci et al., 1976; Baetens et al., 1979), human (Gersell et al., 1978; Paulin and Dubois, 1978; Malaisse-Lagae et al., 1979; Rahier et al., 1979, 1981), dog (Gersell et al., 1978), horse (Furuoka et al., 1989), cat (Furuzawa et al., 1992) and gerbil (Takeuchi et al., 1994). In addition, the following classifications of islets have been reported in the literature: bovine islets have been divided into two types, large and small, in terms of the diameter and location of islets (Bonner-Weir and Like, 1980); rabbit islets have been divided into three types, namely, poly-, bi- and mono-cellular, in terms of cellular composition (Jörns et al., 1988); and dog islets have been classified into two types, namely, poly- and mono-cellular, with the mono-cellular islets being made up of only B-cells (Redecker et al., 1992). In the bovine pancreas we found, however, no mono-cellular islets made up of B-cells exclusively. It has been reported that differences in the size or area and cellular composition of islets exist among and within species (Bonner-Weir and Like, 1980; Jörns et al., 1988; Redecker et al., 1992). The larger is the size or the area of islets, the greater is the number of cell types. For example, in the rabbit (Jörns et al., 1988), small islets are mono-cellular, consisting of B-cells, while larger islets are poly-cellular, being made up of four types of islet cell. In the cows studied here, the area of the islets in the B-region was significantly smaller than that in the A-region and the B-region was completely devoid of A-cells.

Previous reports have revealed a marked difference in the relative numbers of PP- and A-cells in different regions of the pancreas in the rat (Orci et al., 1976; Baetens et al., 1979), human (Gersell et al., 1978; Paulin and Dubois, 1978; Malaisse-Lagae et al., 1979; Rahier et al., 1979, 1981), dog (Gersell et al., 1978), horse (Furuoka et al., 1989), cat (Furuzawa et al., 1992) and gerbil (Takeuchi et al., 1994). Thus, PP-cells appear to be derived from the cells of the ventral primordium, as suggested previously (Orci et al., 1976; Gersell et al., 1978; Paulin and Dubois, 1978; Baetens et al., 1979; Malaisse-Lagae et al., 1979; Rahier et al., 1979, 1981; Furuoka et al., 1989; Furuzawa et al., 1992; Takeuchi et al., 1994). The present study also showed a distinct difference in the size of the PP-cell population between the B- and A-regions.

In the bovine pancreas, the morphological characteristics of the islets in the right lobe and body are the same as those of the islets in the left, which originate from the dorsal pancreatic primordium. The islets in the regions of the body and right lobe did, however, include basically the same components in terms of the endocrine cells as those observed in the uncinate process, which originates from the ventral primordium.

The existence of the various regions in the bovine pancreas suggests that the body and right lobe represent embryologically a region of extensive intersection of both dorsal and ventral pancreatic primordia.

References


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Explanation of Figures

Plate I

Fig. 2a–d. Four serial sections of a pancreatic islet in the left lobe, showing (a) A-, (b) B-, (c) D-, and (d) PP-cells. Note A-, D-, and PP-cells at the periphery and B-cells in the center of the islet. Counterstained with Mayer's hematoxylin. ABC method. ×200. See text for abbreviation.
Plate II

Fig. 3a–d. Four serial sections of a pancreatic islet in the uncinate process, showing (a) A-, (b) B-, (c) D-, and (d) PP-cells. Note random distribution of PP-cells and being devoid of A-cells. Counterstained with Mayer's hematoxylin. ABC method. ×200. See text for abbreviation.
Plate III

Fig. 4. PP-cells of the exocrine parenchyma in the left lobe. Counterstained with Mayer's hematoxylin. ABC method. ×550. See text for abbreviation.

Fig. 5a, b. Two serial sections of the right lobe, showing (a) A-cells and (b) PP-cells. The upper part of each section is the ventral portion; the lower part of each section is the dorsal portion. Note that the ventral portion contains A-cell-rich islets (arrows), and the dorsal portion contains PP-cell-rich islets. Counterstained with Mayer's hematoxylin. ABC method. ×70.