Immunocytochemical Component of Endocrine Cells in Pancreatic Islets of Horses

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ABSTRACT. The endocrine cell components in the pancreatic islets of the following 4 pancreatic regions of the horse were investigated by immunohistochemical methods: *lobus pancreatis sinister* (left lobe); *lobus pancreatis dexter* (right lobe); and 2 regions of *Corpus pancreatis* (body), the duodenal lobe which lies along the cranial duodenal flexure and descending duodenum, and the intermediate lobe which is situated around the portal vein. The islets in the left and intermediate lobes contained a central mass of glucagon cells surrounded by insulin cells, a few somatostatin cells and sporadic pancreatic polypeptide (PP) cells. On the other hand, the islets in the duodenal lobe were small in size compared with the other 3 regions, and were predominant in insulin and pancreatic polypeptide (PP) cells, but almost lacked in glucagon cells. These findings suggested that the duodenal lobe was derived from the ventral pancreatic primordium, and the left and intermediate lobes were originated from the dorsal pancreatic primordium. In the right lobe, the composition and distribution of the islet cells were almost the same as those in the left and intermediate lobes, but there were several lobules containing numerous PP cells as seen in the duodenal lobe. —KEY WORDS: endocrine cells, horse, immunohistochemistry, pancreatic islet.


The embryonic pancreas originates from the dorsal and ventral pancreatic primordia, both fuse and develop into the pancreas. In humans, the caudal part of the head region and the uncinate process region are derived from the ventral pancreatic primordium, while the cephalic part of the head region, and the body and tail regions from the dorsal pancreatic primordium [13].

The distribution of pancreatic polypeptide (PP) and other endocrine cells in the pancreas has been investigated in humans [4, 7, 10, 12, 15-17], dogs [1, 2, 6, 7], cats [2], and rats [14]. These reports show that the endocrine cell components of pancreatic islets are variable among the pancreatic regions derived from the different primordia. On the other hand, the cellular arrangement of pancreatic islets in the horse is reported to be different from that of other mammals by electron-microscopical [3, 5] and immunohistochemical [8, 9, 11] studies; a mass of A cells is exclusively located at the center of the islet and surrounded by B cells. However, differences in the endocrine cell components of pancreatic islets among the pancreatic regions are not clarified in horses.

This paper describes the relative populations of the endocrine cell components of the pancreatic islets in the left lobe, right lobe and body of the equine pancreas, and discusses the relationship between these immunohistochemical findings and each pancreatic region developed from the different primordia.
MATERIALS AND METHODS

Three thoroughbred horses (3- and 7-year-old males, and 5-year-old female) were sacrificed under anesthesia (Succin; Yamanouchi, Japan), and small pancreatic tissues were sampled from lobus pancreatis sinister (left lobe), lobus pancreatis dexter (right lobe), and the following 2 regions of corpus pancreatis (body); the duodenal lobe which lies along the cranial duodenal flexure and descending duodenum, and the intermediate lobe which is situated around the portal vein. Tissue blocks were fixed overnight in Bouin's solution at room temperature, and processed routinely for embedding in paraffin. Sections were cut serially at 2 to 4 μm. Immunohistochemical stainings were done by Sternberger's unlabeled peroxidase-antiperoxidase (PAP) method using the Dako PAP kit (Dako Corp., Calif., USA) from a commercial source. The antisera used in this study were 1:300 rabbit anti-human glucagon, 1:200 rabbit anti-human somatostatin and 1:750 rabbit anti-human PP, and 1:300 guinea pig anti-human insulin. After immunostainings, sections were counterstained with Mayer's hematoxylin. To assess the specificity of the immunohistochemical staining, normal rabbit serum was substituted for the specific primary antisera. Controls omitting the primary antiserum for all the immunohistochemical reactions were negative.

To compare the relative frequency of immunoreactive cells in each region of the pancreas, 4 types of cells per 22.5 mm² were summed up and equated to 100%. The value of each type was converted to a percentage of the total.

One hundred ancreatic islets in sections from each pancreatic region were measured to estimate the relative mean size.

RESULTS

Four kinds of immunoreactive cells, glucagon, insulin, somatostatin and PP cells were detected in the islets as well as in the exocrine acini and epithelia of the inter- and intralobular duct. The relative frequencies of those immunoreactive cells in 4 pancreatic regions are shown in Table 1.

In the left and intermediate lobes, numerous glucagon cells were located at the center of pancreatic islets (Fig. 1a) and surrounded by many insulin cells (Fig. 1b). A few somatostatin cells were distributed between the glucagon and insulin cell groups, or among the insulin cells (Fig. 1c). PP cells were found sporadically or singly in the peripheral area of the islets (Fig. 1d). Each of the 4 immunoreactive cells was also detected singly or in small clusters in the exocrine acini and epithelia of the inter- and intralobular duct.

In the duodenal lobe, a few glucagon cells were found in the islets (Fig. 2a). Insulin cells occupied the greater part of the islets (Fig. 2b). Somatostatin cells were scattered singly or in small clusters among the insulin cells (Fig. 2c). PP cells were usually dotted at the periphery of islets (Fig. 2d), but sometimes gathered to form a peripheral layer or clusters in the islets (Fig. 3a, b). In the exocrine acini and epithelia of the inter- and intralobular duct, both insulin and PP cells were found with considerable frequency, somatostatin cells with low frequency, and glucagon cells scarcely.

In the right lobe, the cellular composition of the islets was almost the same as that in the left and intermediate lobes (Fig. 4a, b, c, d). However, lobules containing many PP cells in both islets and exocrine acini were sometimes observed (Fig. 4d). The islets in these lobules were small in size, devoid of glucagon cells and consisted mainly of insulin and PP cells as seen in the islets of the
EQUINE PANCREATIC ENDOCRINE CELLS

Table 1. Distribution (%) of endocrine cells in 4 pancreatic regions

<table>
<thead>
<tr>
<th>Portion</th>
<th>Left lobe</th>
<th>Right lobe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>I</td>
</tr>
<tr>
<td>Islet</td>
<td>30.68</td>
<td>58.28</td>
</tr>
<tr>
<td>Duct</td>
<td>0.41</td>
<td>0.02</td>
</tr>
<tr>
<td>Acinus</td>
<td>0.55</td>
<td>0.98</td>
</tr>
<tr>
<td>Total</td>
<td>31.64</td>
<td>59.28</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Portion</th>
<th>Intermediate lobe</th>
<th>Duodenal lobe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>I</td>
</tr>
<tr>
<td>Islet</td>
<td>31.79</td>
<td>58.05</td>
</tr>
<tr>
<td>Duct</td>
<td>0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>Acinus</td>
<td>0.43</td>
<td>1.64</td>
</tr>
<tr>
<td>Total</td>
<td>32.36</td>
<td>59.71</td>
</tr>
</tbody>
</table>

G: glucagon cell, I: insulin cell, S: somatostatin cell, PP: PP cell

duodenal lobe.

The average diameter of islets in the left, right, intermediate and duodenal lobes was 0.11, 0.12, 0.12 and 0.06 mm, respectively. It was noted that the diameter in the duodenal lobe was about half as much as that of the other 3 regions.

DISCUSSION

In our study, the pancreatic islets in the left and intermediate lobes contained a central mass of glucagon cells surrounded by insulin cells, a few somatostatin cells and sporadical PP cells. These cellular components were similar to that described by other authors in horses [8, 9, 11]. On the other hand, the morphology of the islets of the duodenal lobe was different from that of the other 3 regions, that is, the islets were the smallest in size and consisted of a few glucagon cells, predominant insulin cells and comparatively rich PP cells. Such unique islets in the duodenal lobe have never been demonstrated in the horse, though observed in mice, rats and hamsters [11, 14]. PP cells have been regarded as the cells derived from the ventral primordium, because they were considerably numerous in the posterior part of the head region in the human pancreas [11, 15, 17]. This fact and our findings suggest that the duodenal lobe in the present study was derived from the ventral primordium, and comparable to the posterior part of the head region in the human pancreas. In addition, the left and intermediate lobes in which the islets contained sporadical PP cells are considered as being originated from the dorsal pancreatic primordium.

The relative proportion of PP cells was as high as 70% in the posterior part of the head in the adult human pancreas [16]. Larsson et al. [11] reported that PP cells varied greatly in frequency among different animal species, and low in mice, rats and hamsters, but
Fig. 1. Four serial sections of a pancreatic islet in a left lobe; (a) glucagon cells, (b) insulin cells, (c) somatostatin cells, and (d) PP cell (arrow). Note a single PP cell at the periphery of an islet in (d). PAP method. ×200.
Fig. 2. Four serial sections of a pancreatic islet in a duodenal lobe; (a) a glucagon cell (arrow), (b) insulin cells, (c) somatostatin cells (arrows), and (d) PP cells (arrows). Note a single glucagon cell at the central area of the islet in (a). PAP method. ×400.
extremely high in sheep. Furthermore, they observed in the horse [11] that PP cells were also present in the exocrine parenchyma as well as at the periphery of the islets and almost as numerous as glucagon cells in the tail (left) region. In the present study, however, PP cells were numerous in the right and duodenal lobes.

In the present study, PP cells were about 24% of the endocrine cells in the duodenal lobe originating from the ventral pancreatic primordium, being more numerous than in the other 3 regions. However, the value of PP cells in the duodenal lobe of the horse was low in comparison with that in the posterior part of the head region in the adult human pancreas which was derived from the ventral pancreatic primordium [16]. The relative proportion of insulin cells was about 72% in the duodenal lobe of the horse, while 30% in the posterior part of the head region in the adult human [16]. Thus, the relative proportion of PP and insulin cells in the region originating from the ventral pancreatic primordium was inverted between the horse and human.

A significant difference observed in some mammals including humans is the inverse distribution between PP and glucagon cells [11, 14, 15]; in the regions originated from the dorsal pancreatic primordium, insulin cells occupied the center of the islets and were surrounded by glucagon cells, while in the regions derived from the ventral pancreatic primordium, insulin cells occupying the center were surrounded by a ring of PP cells instead of glucagon cells. In the present study in horses, however, glucagon cells were located at the center of the islets and insulin cells at their periphery in the regions originated from the dorsal pancreatic primordium, while in the duodenal lobe, glucagon cells were very few and insulin cells occupying center were surrounded by a strand of PP cells.

The morphological characteristics of the
Fig. 4. Four serial sections in a right lobe; (a) glucagon cells, (b) insulin cells, (c) somatostatin cells, and (d) PP cells. Note that lobules rich in PP cells lacked in glucagon cells. PAP method. ×40.
islets in the right lobe were almost the same as those in the left and intermediate lobes originating from the dorsal pancreatic primordium. In a few lobules of the right lobe, however, the islets contained the same components of the endocrine cells as those observed in the duodenal lobe originating from the ventral pancreatic primordium. The existence of such lobules suggests that the right lobe is embryologically the converging area of both dorsal and ventral pancreatic primordia.

REFERENCES

要約

ウマの対島の細胞構成に関する免疫組織化学的検索：古岡秀文・伊東久男1・渋田みゆき・師藤隆彦・佐藤博・松倉知敏（北海道大学獣医学部解剖病理学教室，1家畜解剖学教室）—ウマの対島の4部位、すなわち、左葉、右葉、体部（十二指腸部と中間葉）における対島の細胞構成について、glucagon, insulin, somatostatin, PP抗血清を用いて免疫組織化学的に検索を行った。左葉および中間葉の対島は、glucagon 細胞塊が中央に位置し、insulin 細胞が対島の外側に帯状に配置していた。また、somatostatin, PP 細胞が、insulin 細胞の間際あるいは、周囲に散在性に観察された。十二指腸葉の対島では、glucagon 細胞をほとんど欠き、主として insulin 細胞と PP 細胞から構成され、somatostatin 細胞は主に対島の外側に散在性に観察された。これらのことは、十二指腸葉が数個原基に、左葉および中間葉が数個原基に由来することを示唆していた。右葉における対島の構成は、左葉、中間葉の対島のそれと概ね一致していたが、時に十二指腸葉にみられるような glucagon 細胞を欠き、insulin 細胞と PP 細胞が優勢な対島を有する小葉が観察された。