Immunohistochemical Study of the Pancreatic Endocrine Cells of the Hystrix cristata (porcupine)

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SUMMARY
The pancreatic endocrine cells of Hystrix cristata (porcupine) was investigated by Immunocytochemistry. Immunostaining methods have demonstrated immunoreactivity for insulin, glucagon and somatostatin. Spherical to spindle shaped endocrine cells were identified. Insulin immunoreactive cells were present in the central regions with high frequency, and few of these cells were also demonstrated in the mantle zones. Glucagon immunoreactive cells were mainly restricted to mantle zones but few of these cells were also demonstrated in the central zones. However, rare immunoreactive cells were found in the peripheral regions. Somatostatin immunoreactive cells were detected in the mantle cells in the mantle regions and peripheral regions with moderate and rare frequencies, respectively. In addition some somatostatin immunoreactive cells were also demonstrated in the exocrine portions.

Keywords : Immunohistochemistry; insulin; glucagon; somatostatin; pancreatic islets; endocrine cell, porcupine.

Introduction
The rodents (Rodentia), which are the widest order of placental mammals, comprise more than half of the mammals known at present. Porcupine is from Hystricidae family, which constitutes a small group of the order Rodentia [4, 16]. However, our literature investigation showed that there was no information related to immunohistochemical localization of insulin, glucagon and somatostatin in pancreas of Hystrix cristata. The objective of this investigation in the pancreas of Hystrix cristata was, therefore, to improve the knowledge on the localization of endocrine cells and to prospect for functional role of insulin, glucagon and somatostatin.

The pancreatic endocrine system has been widely studied by immunocytochemistry [11, 14, 15, 18]. It is generally known that the pancreas of rodent is subdivided into exocrine and endocrine portions. Digestive enzymes are released from the exocrine pancreas and regulatory hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide (PP) are produced by the endocrine pancreas and are released into blood circulation. The appearance, regional dispersion and relative frequency of the corresponding endocrine cells are studied by histochemistry using immunofluorescence method [19] and immunohistochemistry [26, 27]. In addition, investigations of gastroenteropancreatic (GEP) endocrine cells are considered to be an important part of phylogenetic study [3, 20].

The pancreas has been treated as a valuable organ for endocrine studies and endocrine pancreas has been extensively studied in association with diabetes [6, 8]. Until now, the regional distribution and the relative frequency of four major endocrine immunoreactive cell types (insulin, glucagon, somatostatin and pancreatic polypeptide (PP)) have been reported in the pancreas of the rodents such as hamster [2], sand rat [5], C57BL/6 mouse [6], guinea pig [22], gerbil [14], teleosts [17], shark [10] and wood Mouse [29].

It has been classically admitted that insulin immunoreactive cells are located in the central regions and that the other (glucagon and somatostatin) immunoreactive cells are located in the peripheral or mantle zones. But, many researches suggested that the species-dependent characteristic distribution of endocrine cells could result from feeding habits [28]. Although many studies were conducted on various vertebrates including several species and strains of rodents, no report about immunohistochemistry of pancreatic endocrine cells of the Hystrix cristata was available. The purpose of the present study was to clarify the regional distribution and the relative frequency of endocrine cells in the pancreatic islets of the porcupine, Hystrix cristata, using an immunohistochemical method (ABC) and three types of specific

RÉSUMÉ
Etude immunohistochimique des cellules endocrines pancréatiques chez le Porc-épic (Hystrix cristata). Par SEMA TIMURKAAN, AYDIN GIRGIN, MERYEM KARAN


antibodies against insulin, glucagon, and somatostatin respectively.

**Materials and methods**

Six adult porcupines were anesthetized and killed using ether. The pancreas was removed immediately and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7.4, for 18hrs before paraffin embedding. Tissues were routinely processed through a graded series of alcohols, cleared in xylol and embedded in paraffin. 5μm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out by the peroxidase linked avidin-biotin complex (ABC) method. Blocking of endogenous peroxidase activity was carried out with % 0.08 hydrogen peroxidase (H₂O₂) in methanol for 5 minutes [27]. In order to the block unspecified binding, an incubation with normal goat serum (1:10) in 0.1 M PBS, pH 7.2 was performed. Sections were incubated for 16-20 hrs at 4°C in mouse anti-insulin IgG (Sigma), anti-glucagon IgG (Sigma) and anti-somatostatin IgG antibodies. The antibodies were diluted to 1:500 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin. Sections were then incubated in biotinylated sheep anti-mouse IgG (Sigma) and with streptavidin horseradish peroxidase (Dako), both at a dilution of 1:50 in PBS, for 1 hr at room temperature. Sections were washed in PBS for 30 minutes after each incubation. Sections were then immersed in glucose oxidase-DAB-nickel ammonium sulphate (GDN) substrate [25] for 10 minutes, washed in distilled water and counterstained with eosin. Sections were examined with light microscope and photomicrographs were taken.

Negative controls were made by replacing each specific antiserum with normal rabbit or goat serum. No immunoreactive structures were observed.

Mean numbers of immunopositive cells in each sample obtained from pancreas were determined by counting the positive cells in five randomly selected microscopic fields with 40X magnification (numbers of immunopositive cells/microscopic fields). Then the arithmetic means and standard errors were calculated for each sample.

**Results**

In this study, three types of immunoreactive endocrine cells were detected using antisera to insulin, glucagon and somatostatin in the pancreas of the *Hystrix cristata*. The pancreatic islets were distinctly divided into three distinct layers: central, mantle and peripheral regions. Different distributions and frequencies of the immunoreactive endocrine cells were recorded according to the pancreatic regions (Table 1). Endocrine cells were mainly spherical to spindle or occasionally oval to round-shaped.

Insulin immunoreactive cells were detected with a high frequency in the central regions of the pancreatic islets, and more scarcely in the mantle zones (Table 1, Fig. 1). No insulin positive cells were found in the peripheral regions or in the exocrine portions. In the central zones, cells were mainly spherical to spindle shaped and round to oval forms of variable size were rarely observed. In addition, few cells were also observed in mantle zone intermingled with other immunoreactive cells, especially glucagon and somatostatin immunoreactive cells.

Glucagon immunoreactive cells were mainly restricted to mantle zones but some positive cells were also encountered in the central zones (Table 1, Fig 2). However, rare examples were found in the peripheral regions and, no glucagon immunoreactive cells were found in the exocrine portions.

Somatostatin immunoreactive cells were mainly detected in the mantle zones and in the peripheral regions at a lesser extend (Table 1). Few somatostatin endocrine cells were also scattered in the central regions of the pancreatic islets (Fig 3) and in the exocrine portions (Fig 4).

**Discussion**

In the pancreas of the porcupine, *Hystrix cristata*, all of the three major endocrine cell types were distributed through the endocrine pancreas. All these endocrine cells appeared mainly spherical to spindle forms.

Insulin is synthesized in the B cells of the pancreatic islets and regulates the serum glucose concentrations [7]. In the mammals, the regional distribution and the relative frequency of pancreatic insulin immunoreactive cells were reported in the hamster [2], C57BL/6 mouse [5], gerbil [14], wood Mouse [29], voles [24], opossum [12] and various laboratory animals [28]. From these reports, it is well established that insulin immunoreactive cells are located in the central regions of mammalian pancreatic islets and that the other cells, glucagon and somatostatin immunoreactive cells, surrounded them. However, REDY et al [23] reported that these immunoreactive cells are observed in the majority of islets where they occur peripherally as groups of cells, and within the pancreatic islets of several marsupial species. In the pre-

<table>
<thead>
<tr>
<th>Immunoreactive cells</th>
<th>Pancreatic islet portions</th>
<th>Exocrine portion</th>
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<tbody>
<tr>
<td></td>
<td>Central</td>
<td>Mantle</td>
</tr>
<tr>
<td>Insulin</td>
<td>9.28 ± 0.48</td>
<td>6.55 ± 0.56</td>
</tr>
<tr>
<td>Glucagon</td>
<td>6.45 ± 0.37</td>
<td>10.28 ± 1.15</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>2.18 ± 0.72</td>
<td>11.25 ± 0.97</td>
</tr>
</tbody>
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---: not detectable

**Table 1.** — Numbers of insulin, glucagon and somatostatin immunoreactive cells in the pancreas of the *Hystrix cristata*. Results are expressed as arithmetic means ± standard errors of five randomly selected microscopic fields with 40X magnification.

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In the present study, most of the insulin immunoreactive cells were restricted to the central regions of islets in the *Hystrix cristata*, which is similar to previous reports on rodents [2, 6, 26, 28, 29].

Glucagon is synthesized in the A cells of the pancreas and also participates in the regulation of blood glucose concentrations [7]. Morphologically similar cells are also present in the digestive tract of the dog [28]. In the present study, glucagon immunoreactive cells were mainly restricted to mantle zones but some of these cells were also present in the central zones of the porcupine pancreas. These results were found to be similar to that of mammalian pancreatic islets [2, 6, 12, 24, 28]. Cell clusters consisted of glucagon immunoreactive cells located in the connective tissue regions of pancreatic duct portions are generally detected in higher mammals [13], but these cellular structures were seldom in rodents.

Somatostatin was isolated from hypothalamus of sheep for the first time and it could be occurred into straight and cyclic forms [1]. This substance inhibits the secretion of the gastrin, cholecystokinin, secretin, glucagon, insulin, motilin and gastric acid [9] and the absorption of amino acid, glucose and fatty acids in the gastrointestinal tract [21]. In the porcupine pancreas, somatostatin immunoreactive cells were principally detected in the mantle zones, then in the peripheral regions and rarely in the exocrine portions. This distribution is partially comparable with previous reports that have evidenced somatostatin positive cells in the outer-

**Figure 1.** — Insulin-immunoreactive cells in the pancreatic islets of *Hystrix cristata*. Insulin-positive cells appear as intensely labelled, dark cells. 20 x 5.

**Figure 2.** — Glucagon-immunoreactive cells in the pancreatic islets of *Hystrix cristata*. 20 x 5.

**Figure 3.** — Cells containing somatostatin were scattered throughout the islets. 20 x 5.

**Figure 4.** — Somatostatin immunoreactive cells were also demonstrated in the exocrine portions. 20 x 5.
most regions of mammalian pancreatic islets [2, 6, 12, 24, 28, 29]. However, in the present study, most of these immuno-reactive cells found in the mantle zones were mixed with glucagon immuno-reactive cells. These topographic findings differ from distributional patterns previously reported in other mammalian species [2, 6, 12, 24, 28, 29].

In conclusion, the regional distribution of endocrine cells in the pancreatic islets of *Hystric cristata* was roughly similar to that of other mammals, especially rodents, except for the topographically different distribution of somatostatin endocrine cells.

References