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An immunohistochemical study of the pancreatic endocrine cells in the cat-shark, *Scyliorhinus torazame*

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두툽상어 췌장에 출현하는 내분비세포의 면역조직화학적 연구

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초록 : 두툽상어의 췌장 내분비세포를 면역조직화학적으로 관찰하였던바, 5종의 면역반응세포들이 동정되었다.

glucagon 면역반응세포는 도관의 상피간 및 췌장외분비선의 선포주위에서 한개 또는 다수의 집단으로 출현하였으며, insulin 면역반응세포는 islet의 주변부 또는 도관의 상피간에 각각 관찰되었다. somatostatin 면역반응세포는 도관의 상피간 또는 췌장외분비선의 주위에 한개 또는 집단으로 분포하였으며, 소수의 5-HT 면역반응세포는 islet의 주변부 및 췌장 외분비선의 선세포간에서 관찰되었다. BPP 면역반응세포는 극소수로 관찰되었으며, GAS/CCK와 chromogranin 면역반응세포들은 관찰되지 않았다.

Key words: pancreatic endocrine cells, cat-shark, immunoreactive cell.

Introduction

It has been well known that the exocrine and endocrine and endocrine cells producing digestive enzymes and regulatory peptides, respectively, are found in the pancreas of various vertebrates. Recently, the distribution, shapes and types of the pancreatic endocrine cells have been intensively investigated in many animals[1~16], using both electron microscopic and immunohistochemical techniques. Although a number of reports have undergone in the pancreatic endocrine cells of fishes[10~16], among them only a few papers have been carried out on those of elasmobranchii[11,13]. In the Shark, *Scyliorhinus stellaris*, Kobayashi and Ali[13] reported on the eight cell types and their ultrastructure in the endocrine pancreas. In the present study, the endocrine cells of the pancreas in an elasmobranchii species, *S. torazame*, have been studied with special reference to their types and distributions by means of an immunohistochemical method.

Materials and Methods

Five cat-sharks, *S. torazame* of both sexes collected at Pusan in Korea, were immediately dissected. Pancreatic tissues were fixed in Bouin's fluid and embedded in paraffin. The serial sections were cut at 3~4μm thickness. Representative deparaffinized and dehydrated sections were stained with the peroxidase-antiperoxidase(PAP) method[17] for identifing specific pancreatic endocrine cells. Details of the antisera used are shown in the Table 1. After staining, each section was lightly counterstained with Mayer's hematoxylin, dehydrated, cleared in

Table 1. Properties of antisera used

Antisera raised[a)]	Code	Source	Dilution
Glucagon	8635013	Immuno Nuclear Corp. (INC), Stillwater	1 : 800
Insulin	8622014	INC	1 : 2,000
Somatostain	CA325	Cambridge Research Biochemical (CRM), Billerica	1 : 1,000
5-HT	8535028	INC	1 : 10,000
Pancreatic polypeptide	i607	UCB-bioproducts	1 : 5,000
Gas/CCK	i600/004	Union Chimique Belge (UCB) bioproducts	1 : 100
Chromogranin	8541012	INC	1 : 2,000

a) All antisera were raised in the rabbits, except that against insulin, which was raised in a guinea pig. 5-HT: 5-hydroxytryptamine, Gas/CCK: Gastrin/Cholecystokinin

xylene and mounted.

Results

The pancreatic endocrine cells of *S. torazame* were showed elongated large islets and single or small groups among the exocrine acinar cells or closely related to the interlobular ducts.

Numerous glucagon-immunoreactive cells were spherical or spindle in shape, and scattered in the whole large elongated islets and the epithelia of the interlobular duct. A few round or polygonal shaped cells were distributed between the pancreatic acini (Figs 1a-d).

Moderate insulin-immunoreactive cells were distributed in the epithelia of the interlobular duct or in the periphery of the islet. Most of them were spherical or polymorphic in shape, and clustered in while small groups in the periphery of the pancreatic islet a few were located in pancreatic acini (Figs 2a-d).

Moderate somatostatin-immunoreactive cells were spherical or spindle in shape, and occupied in the epithelia of the interlobular duct or in the periphery of the pancreatic islet (Figs 3a-d).

A few 5-HT-immunoreactive cells were exclusively found in the periphery of the pancreatic islet. These cells were spindle in shape (Figs 4a, b).

Discrete round-shaped BPP-immunoreactive cell was observed in the periphery area of the pancreatic islet. The granules of immunoreactive cell was poorly stained.

In the present study, GAS/CCK- and chromogranin-immunoreactive cells were not detected at any sites throughout the pancreas of this species.

Discussion

The pancreas in teleosts is a diffuse organ widely extended within the mesentery, and the pancreatic endocrine cells are clustered in islets relatively isolated from the exocrine gland; on the other hand, the pancreatic endocrine cells of cartilagenous fishes have a close relationship to the excretory duct[13,16].

The pancreatic endocrine cells of *S. torazame* were quite similar in appearance to the shark, *S. stellaris* reported by Kobayashi and Ali[13].

Using seven antisera of mammals peptide hormones, we found five cell types in pancreas of cat-shark, *S. torazame*. Most of these immunoreactive cells were located in the epithelia of interlobular duct and throughout the pancreatic islet, while a few immunoreactive cells in the acini of the pancreas.

Glucagon-immunoreactive cells were found in the periphery of islet in the various animals[2~16] except horse[1], while in the present study, they were detected in the whole pancreatic islet and epithelia of the interlobular duct or few between the pancreatic acini.

Also insulin-immunoreactive cells of reptiles[7,8], amphibian[9] and many fishes [10~16] were found in the central area of the islet, while most of this study in the periphery.

In this study, somatostatin-immunoreactive cells were reminiscent of horse[1] and teleosts[15] which observed in the periphery of the pancreatic islet in addition to the epithelia of the interlobular duct.

5-HT-immunoreactive cells, in this study, were located exclusively in the periphery of the islet including vampire bat[3], caiman[8] and teleosts[15], while

in the most animals[1,2,4-7,9-14,16] were not encounted.

In this study, the pale reaction of the granules in the BPP-immuuoreactive cell was assumed due to differences in protein molecular structure.

Chromogranin- and GAS/CCK-immunoreactive cells of *Storazame* were not seen similarly to the other animals[1,16].

The morphological characteristics were consistent with those of previous reports having from mainly spherical to polymorphic shaped. But, the discrepancy of the distributions in pancreatic endocrine cells was probably considered concerning with their environments, physiological characteristics and diet differences among the species.

Summary

The pancreatic endocrine cells of the cat-shark, *S. torazame*, were studied using immunohistochemical method. Five kinds of endocrine cells (glucagon-, somatostatin-, insulin-, 5-HT- and BPP-immunoreactive cells) identified in this study. The chracteristic findings of the distributions of five immunoreactive cells were as follows.

Glucagon-immunoreactive cells were detected as clustering group in the epithelia of the interlobular duct and singly the pancreatic acini, respectively. Insulin-immunoreactive cells were moderately observed in the epithelia of the interlobular duct or in the periphery of the islet. Somatostatin-immunoreactive cells were distributed in single or mass groups in the epithelia of the interlobular duct and the exocrine gland of the pancreas. A very few 5-HT-immunoreactive cells were seen in the periphery of the islet and the acini of the pancreas. BPP-immunoreactive cell was singly located in the periphery of the pancreatic islet, but GAS/CCK- and Chromogranin-immunoreactive cells were not found in this study.

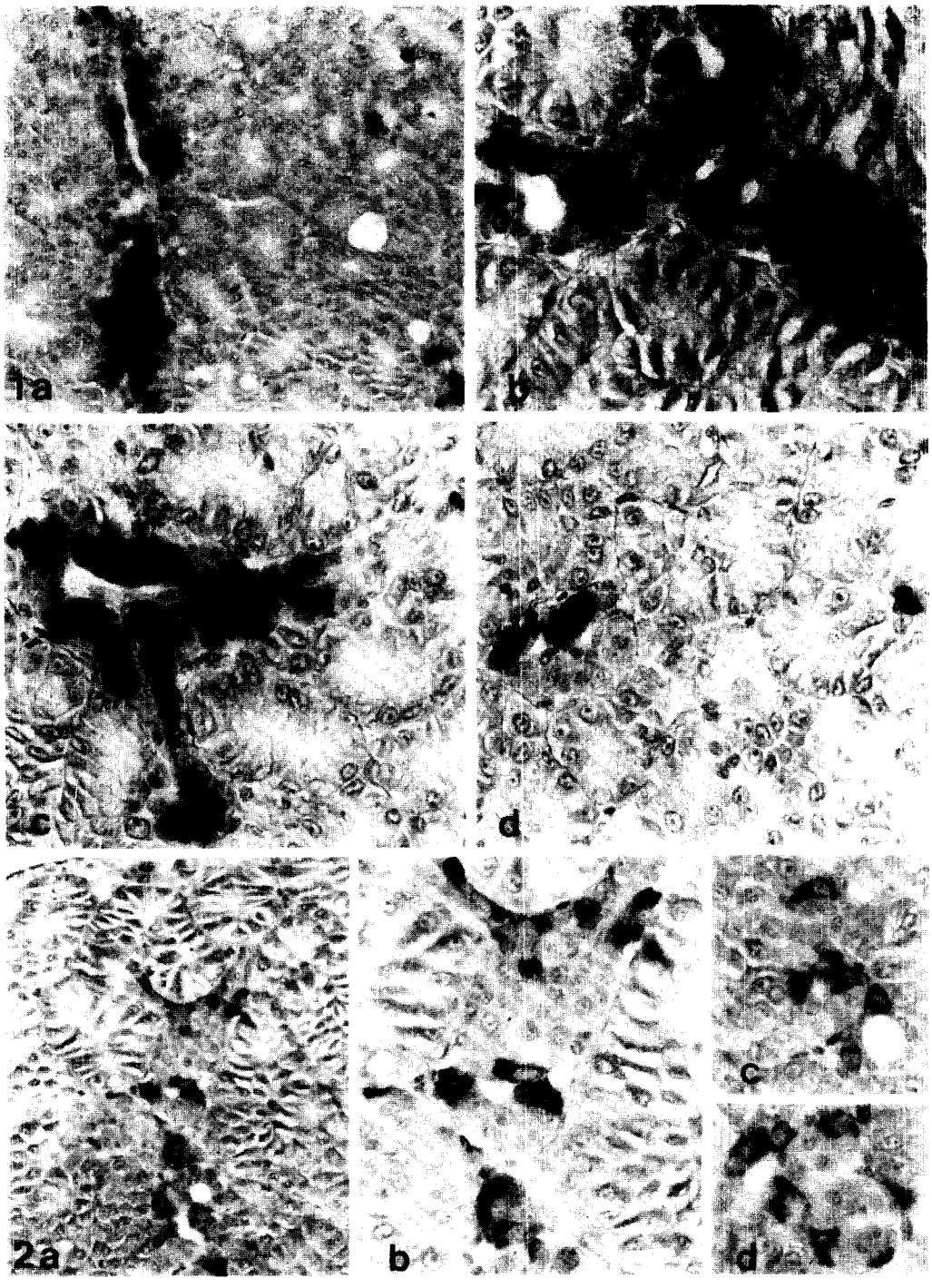
Legends for figures

Figs 1a-d: (a-c) Glucagon-immunoreactive cells were detected in the epithelia of the interlobular duct and (d) in the periphery of the pancreatic acini.
a: ×150, b-d: ×300

Figs 2a-d: (a-d) Insulin-immunoreactive cells were observed in the epithelia of the interlobular duct or the periphery of the islet.
a: ×150, b-d: ×300

Figs 3a-d: (a-d) Somatostatin-immunoreactive cells were seen in the epithelia of the interlobular duct and in the exocrine gland of the pancreas.
a: ×150, b,d: ×300, c: ×600

Figs 4a, b: (a) 5-HT-immunoreactive cells were seen in the periphey of islet and (b) the acini of the pancres.
a: ×300, b: ×600


1a
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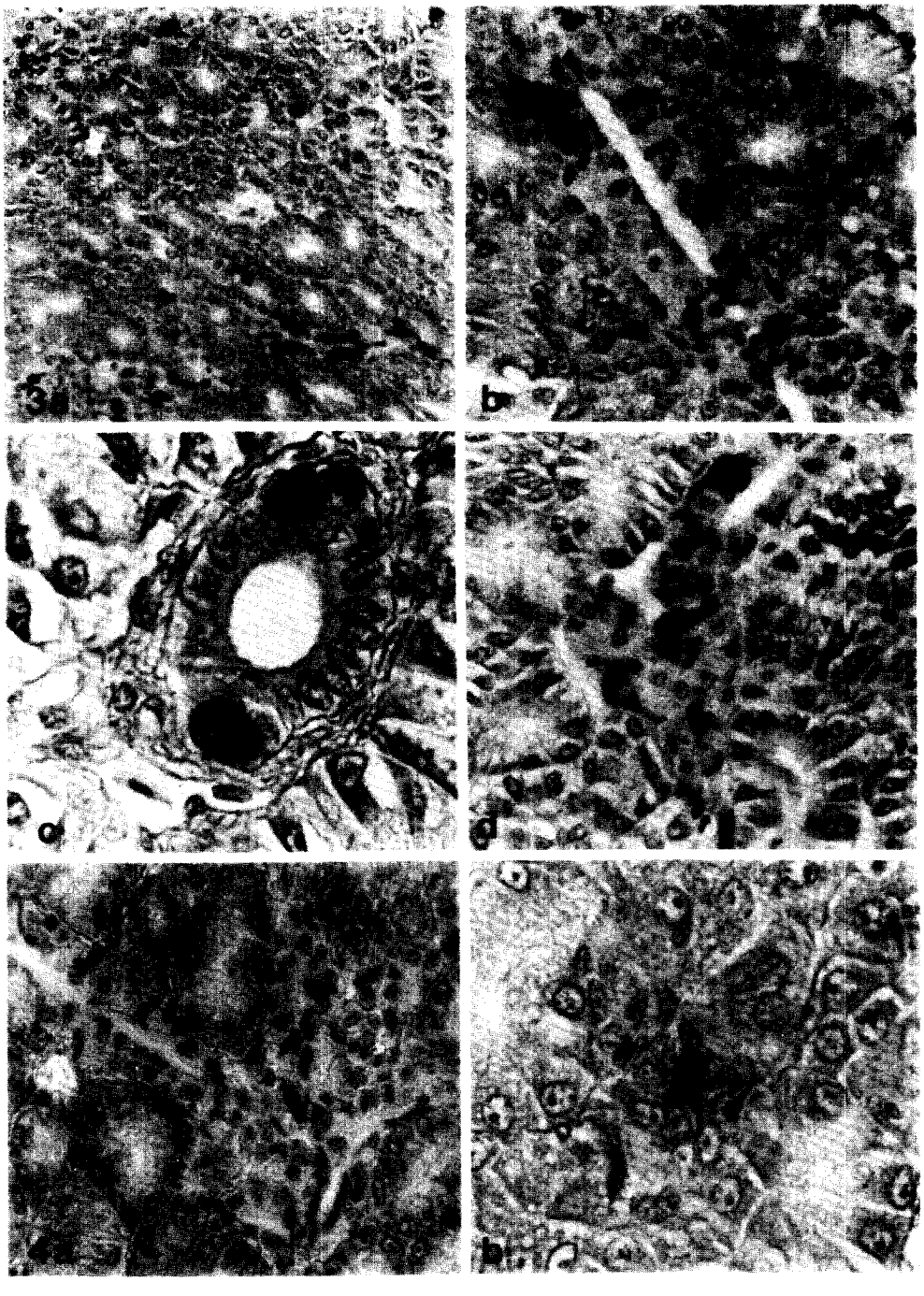

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