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An immunohistochemical study on the pancreatic islets cells of the Mongolian gerbils, *Meriones unguiculatus*

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In order to study the regional distribution and relative frequency of the immunoreactive endocrine cells in the pancreatic islets of the Mongolian gerbil, pancreatic sections of *Meriones unguiculatus* were immunostained using an immunohistochemical (PAP) method with four types of specific antisera against insulin, glucagon, somatostatin and human pancreatic polypeptide (PP). The pancreatic islets were subdivided into three portions (central region, mantle zone and peripheral region) according to their composition of immunoreactive cells. Spherical to spindle shaped insulin, glucagon, somatostatin and PP-immunoreactive cells were observed in this study. Insulin-immunoreactive cells were present in the central regions with high frequency, and a few of these cells were also demonstrated in the mantle zones. Glucagon-immunoreactive cells were mainly restricted to the mantle zones. However, rare examples were found in the peripheral regions. As for the glucagon-immunoreactive cells, somatostatin-immunoreactive cells were detected in the mantle zones and peripheral regions with moderate and rare frequencies, respectively. PP-immunoreactive cells were found in the mantle zones and peripheral regions with rare and moderate frequencies, respectively. In the mantle and the peripheral regions, cytoplasmic process of glucagon-, somatostatin- and PP-immunoreactive cells were intermingled. In conclusion, the regional distribution of endocrine cells in the pancreatic islets of Mongolian gerbil was found to be similar to that of other mammals, especially other rodents, except for the topographical different distribution of somatostatin which differs that of other rodents.

Key words: Mongolian gerbil, pancreatic islets, endocrine cell, immunohistochemistry

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Introduction

It is generally known that the pancreas of vertebrates is subdivided into exocrine and endocrine portions. Digestive enzymes are released in the exocrine and regulatory hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide (PP) are produced in the endocrine and released into blood circulation. The appearance, regional distribution and relative frequency of these regulatory hormones secreted by endocrine cells in the pancreas are well recognized by their histochemistry using [17], the immunofluorescence method [23] and immunohistochemistry [32]. In addition to the above regulatory hormones, peptide YY-, neuropeptide YY- [1], chromogranin family- [14, 27] and motilin- [35] immunoreactive cells have also been demonstrated in the vertebrate pancreas. The pancreas has been treated as a valuable organ for endocrine study and the endocrine pancreas has been extensively studied in association with diabetes [15]. In addition, investigations of gastroenteropancraetic (GEP) endocrine cells are considered to be an important part of phylogenetic study [6].

The Mongolian gerbil, *Meriones unguiculatus*, is a rodent of the family Cricetidae, although it has been included alternatively among the Muridae. The animal is an active, nearly ordorless, usually nonaggressive rodent distinguished by its monogamous mating behavior, water and temperature conservation mechanisms, spontaneous epileptiform seizures, relative freedom from spontaneous disease, and several other unique attributes of interest in research [11].

Until now, the regional distribution and relative frequency of four major immunoreactive cells, insulin-, glucagon-, somatostatin and PP-, have been reported in the pancreas of the hamster [3], sand rat (*Psammomys obesus*) [8], C57BL/6 mouse [10], herbivorous Japanese field vole (*Microtus montebelli*) [22], guinea pig [25], vole (*Microtus arvalis*) [28], obese ob+/ob+ mouse [30], preobese and obese yellow Avy/- mice [33] and wood mouse (*Apodemus speciosus*) [36]. In addition, angiotensin II-immunoreac-

tive cells were found in the pancreas of the mouse [20] and the appearance of calcitonin gene-related peptide- and cholecystokinin-immunoreactive cells have been reported in the rat pancreas [7, 29]. With the increasing demand for diabetic animal models in many fields, the regional distribution and relative frequency of pancreatic endocrine cells, especially, insulin- and glucagon-producing cells in laboratory animals is of interest [9, 10, 33]. It has been accepted that insulin-immunoreactive cells are located in the central regions and that the other immunoreactive cells such as glucagon-, somatostatin- and PP-immunoreactive cells are located in the peripheral or mantle zones. But, many researchers have suggested that species-dependent hormone producing cell distributions in the pancreas of different species might due to feeding habits, and this is now generally accepted [34]. In addition, it has also been reported that different regional distributions and relative frequencies of endocrine cells in the pancreatic islets were demonstrated in different portion of the pancreas, which included the pancreas of single animals [36] and strain-dependent characteristic distributions of these immunoreactive cells was also detected in connection with attempts to increase the production of genetically mutated laboratory animals, and to increase the breeding rate of laboratory animals having specific diseases or unique characteristics, especially in the rat and mouse [9, 10, 30, 33, 36].

Although many studies have concerned the regional distribution and relative frequency of different endocrine cells in the pancreas of various vertebrates including several species and strains of rodents, there have been no reports on immunohistochemical studies into the endocrine cells of the pancreatic islets of the Mongolian gerbil, in spite of their biological, physiological and anatomical differences from the other rodents. The purpose of the present study was to clarify the regional distribution and relative frequency of endocrine cells in the pancreatic islets of the Mongolian gerbil, *Meriones unguiculatus* using an immunohistochemical method (PAP method) and four types of specific antisera against insulin, glucagon, somatostatin and PP.

Materials and Methods

Five adult (40~50 g of body weight) Mongolian gerbils, *Meriones unguiculatus*, were acquired from the Asan Institute for Life Science (Seoul, Korea) and were used in this study without sexual distinction. After food restriction for 24 hours, the animals were anesthetized with ethyl ether and then phlebotomized. Samples from the pancreas were fixed in Bouin's solution, and after paraffin embedding, 3-4 μm serial sections were prepared. Representative sections of each tissue were stained with hematoxylin and eosin for light microscopic examination of the normal gastrointestinal architecture.

Each representative section was deparaffinized, rehydrated and immunostained by the peroxidase anti-peroxidase (PAP) method [31]. Nonspecific reactions were blocked with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsing in phosphate buffered saline (PBS; 0.01 M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS buffer and finally the PAP complex was prepared. The peroxidase reaction was carried out in a solution of 3,3'-diaminobenzidine tetrahydrochloride, containing

Table 1. Antisera used in this study

Antisera raised*	Code	Source	Diluton
Insulin	PUO290395	BioGenex Lab., San Ramon.	1 : 20
Glucagon	PUO390598	BioGenex Lab., San Ramon.	1 : 20
Somatostatin	PUO421295	BioGenex Lab., San Ramon.	1 : 20
PP[1)]	PUO660495	BioGenex Lab., San Ramon.	1 : 20

*All antisera were raised in rabbits except for insulin, which was raised in rabbits.

[1)] PP: human pancreatic polypeptide

Table 2. Regional distributions and relative frequencies of the endocrine cells in the pancreatic islets of the Mongolian gerbil, *Meriones unguiculatus*

Immunoreactive cells	Pancreatic islets		
	Central region	Mantle zone	Peripheral region
Insulin	+++	+	-
Glucagon	-	+++	±
Somatostatin	-	++	±
PP[1)]	-	±	++

* Relative frequencies; +++: numerous, ++: moderate, +: a few, ±: rare, -: not detected

[1)] PP: human pancreatic polypeptide.

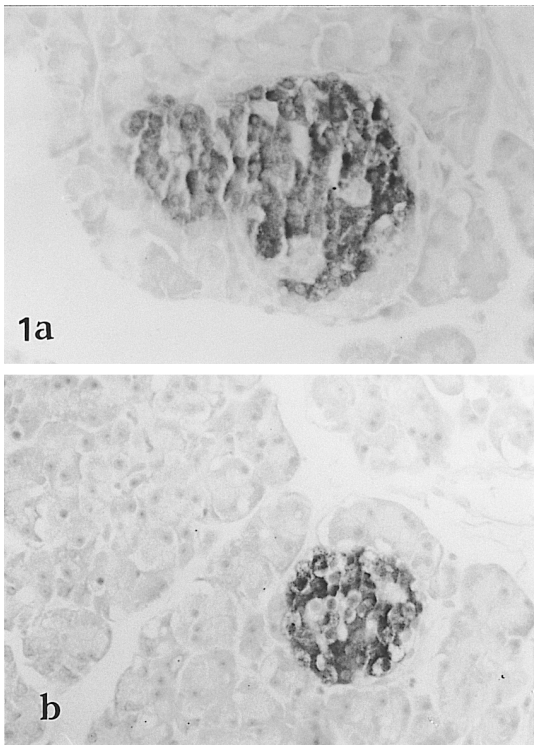

Fig. 1. Insulin-immunoreactive cells in the pancreatic islets of Mongolian gerbils. Note that most of the immunoreactive cells were located in the central regions of pancreatic islets regardless of size. a, b: ×240. PAP method.

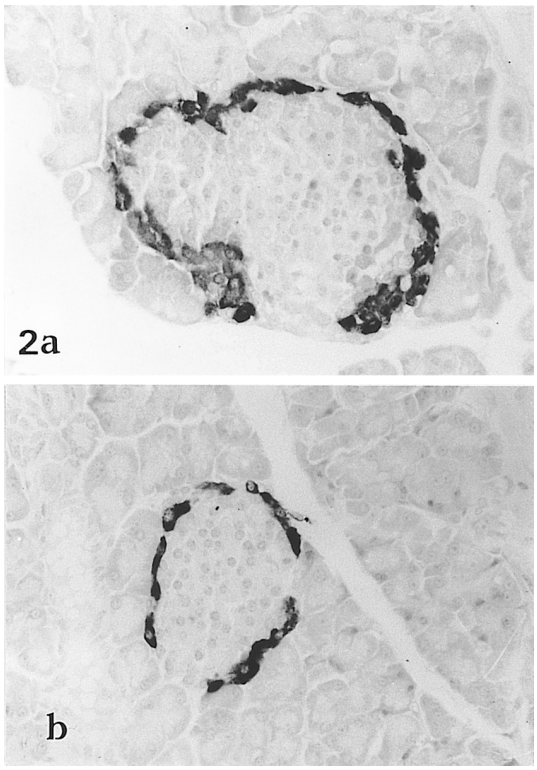

Fig. 2. Glucagon-immunoreactive cells in the pancreatic islets of Mongolian gerbils. Note that most of the immunoreactive cells were located in the mantle zones of pancreatic islets regardless of size. a, b: ×240. PAP method.

0.01% H_2O_2 in Tris-HCl buffer (0.05M, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and immunoreactive cells were observed under a light microscope.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger [31], including the replacement of specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen. The relative frequency of occurrence of each type of immunoreactive cell was allocated to one of five categories according to its frequency, as observed by light microscopy.

Results

In this study, all four kinds of the immunoreactive endocrine cells were detected using antisera against insulin, glucagon, somatostatin and PP in the pancreatic islets, which were distinguished as three distinct layers, a central region, a mantle zone and a peripheral region with their composition of immunoreactive cells. Different regional distributions and relative frequencies of these immunoreactive cells were observed in the different pancreatic regions, and these differences are shown in Table 2. Spherical to spindle or occasionally oval to round-shaped immunoreactive cells were observed in the pancreatic islets of the Mongolian gerbil.

Insulin-immunoreactive cells

Spherical to spindle shaped insulin-immunoreactive cells were located in the central pancreatic islet region with numerous frequency and rarely round to oval shaped cells of variable size were also observed. In addition, a few frequencied cells were also observed in the mantle zone intermingled with other immunoreactive cells, especially glucagon- and somatostatin-immunoreactive cells. However, no insulin-immunoreactive cells were found in the peripheral regions, which predominantly contained PP-immunoreactive cells (Fig. 1a, b).

Glucagon-immunoreactive cells

Spherical to spindle shaped glucagon-immunoreactive cells were located in the mantle and peripheral regions of the pancreatic islets with numerous and rare frequencies, respectively, regardless of size. Occasionally, rare round to ovally shaped cells were also observed in these regions. In the mantle and peripheral regions, the cytoplasmic processes of glucagon-immunoreactive cells were intermingled with other immunoreactive cells especially

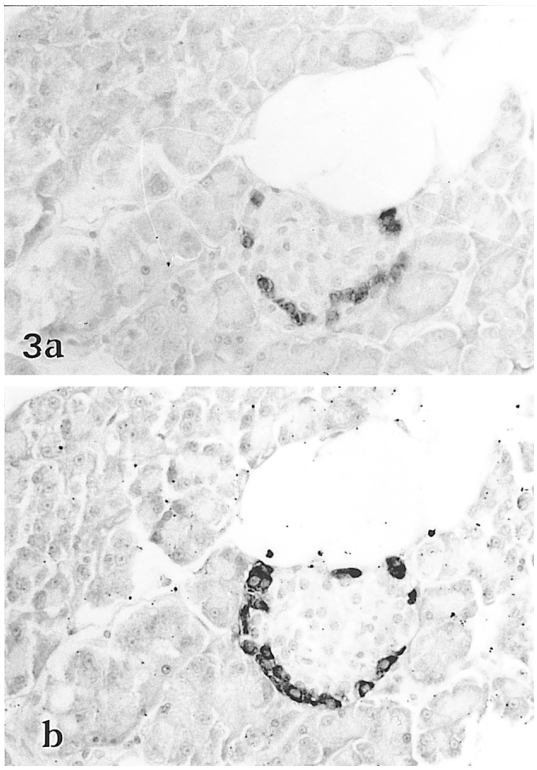

Fig. 3. Somatostatin (a)- and human pancreatic polypeptide (b)-immunoreactive cells in the pancreatic islets of Mongolian gerbils. Note that most of the somatostatin-immunoreactive cells were located in the mantle zones of pancreatic islets regardless of size while human pancreatic polypeptide-immunoreactive cells were observed in the outermost peripheral regions of pancreatic islets. a, b: ×240. PAP method.

somatostatin- and PP-immunoreactive cells. However, no glucagon-immunoreactive cells were observed in the central regions, where numerous insulin-immunoreactive cells were found (Fig. 2a, b).

Somatostatin-immunoreactive cells

Spherical to spindle shaped somatostatin-immunoreactive cells were found in the mantle and peripheral regions of the pancreatic islets with moderate and rare frequencies, respectively, regardless of size. Occasionally, rare round to ovally shaped cells were also observed in these regions. In the mantle and peripheral regions, the cytoplasmic processes of these immunoreactive cells were intermingled with other immunoreactive cells, especially glucagon- and PP-immunoreactive cells. However, no somatostatin-immunoreactive cells were observed in the central regions where numerous insulin-immunoreactive cells were found (Fig. 3a).

PP-immunoreactive cells

Spherical to spindle shaped somatostatin-immunoreactive cells were observed in the mantle and peripheral regions of the pancreatic islets with rare and moderate frequencies, respectively, regardless of their size. Occasionally, rare round to ovally shaped cells were also found in these regions. In the mantle and peripheral regions, the cytoplasmic processes of PP-immunoreactive cells were intermingled with other immunoreactive cells, especially glucagon- and somatostatin-immunoreactive cells. However, no PP-immunoreactive cells were demonstrated in the central regions, where numerous insulin-immunoreactive cells were found (Fig. 3a).

Discussion

Unlike other rodents, Mongolian gerbils of both sexes have a distinct midventral abdominal pad composed of large sebaceous glands under the control of gonadal hormones [4], and Mongolian gerbil has unique feeding habits [11]. In addition, the male gerbils have higher packed red-cell volumes (PCV), hemoglobin levels, total leukocyte counts, and circulating lymphocyte counts than the females, and some erythrocytes of both sexes show a prominent polychromasia and basophilic stippling [11]. In spite of their biological, physiological and anatomical differences from other rodents, no immunohistochemical studies are available on the pancreatic endocrine cells in the pancreatic islets of the Mongolian gerbil. In the present study, the four major types of endocrine cells, insulin-, glucagon-, somatostatin- and PP-immunoreactive cells that are generally found in the mammalian pancreas, were detected in the pancreatic islets.

Insulin is synthesized in the B cells of the pancreatic islets and regulates the serum glucose levels [13]. In mammals, the regional distribution and relative frequency of insulin-immunoreactive cells in the pancreas have been reported in the hamster [3], three-toed sloth (*Bradypus variegates*) [5], C57BL/6 mouse [10], opossum [18], Australian brush-tailed possum [19], voles [28], various laboratory animals [34] and wood mouse [36]. From these reports, it is known that insulin-immunoreactive cells are situated in the central regions of the mammalian pancreas and that other cells, such as, glucagon-, somatostatin- and PP-immunoreactive cells, surrounded them. However, somewhat contradicting the finding of other researchers, Reddy et al. [26] 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 present study, most of the insulin-immunoreactive cells were restricted to the central regions of islets in the Mongolian gerbil, which is similar to previous reports on rodents [3, 10, 28, 33, 34, 36].

Glucagon is synthesized in the A cells of the pancreas and regulates blood glucose levels [13]. Morphologically similar cells are also present in the digestive tract of the

dog. In the present study, glucagon-immunoreactive cells were found in the mantle and the peripheral regions of pancreatic islets. Although glucagon-immunoreactive cells have been found in the mantle and peripheral regions of mammalian pancreatic islets [3, 5, 10, 18, 19, 28, 33, 34, 36] including the present study, species-dependent variations have been reported in the equine pancreas, in which A-cells, demonstrated by anti-glucagon, were found in the center of pancreatic islets [12]. In addition, it has also been reported that under specific disease conditions, such as, those in the obese (diabetic condition) mouse, glucagon-immunoreactive cells are intermingled with insulin-immunoreactive cells in the central regions of the pancreatic islets. In contrast, normal non-obese littermates showed a peripheral localization of these immunoreactive cells [30].

Somatostatin, which consists of 14 amino acids, was isolated initially from the hypothalamus of sheep. It was found to be present in straight and cyclic forms [2]. This substance inhibits the secretion of gastrin, cholecystokinin, secretin, glucagon, insulin, motilin and gastric acid [16] and the absorption of amino acids, glucose and fatty acids in the gastrointestinal tract [2]. To date, somatostatin-immunoreactive cells have been found in the outermost regions of mammalian pancreatic islets [3, 5, 10, 18, 19, 28, 33, 34, 36]. However, in the present study, most of these immunoreactive cells were found in the mantle zones, mixed with glucagon-immunoreactive cells; PP-immunoreactive cells were found to occupy the outermost regions of pancreatic islets in this study. These topographically different distributional patterns in mammalian species [3, 5, 10, 18, 19, 28, 33, 34, 36] are considered as species-dependent variations, and intra-species topographical variations are considered as reflections of unique diseases and suggests that under specific disease conditions, such as obesity (diabetic condition) mouse, somatostatin-immunoreactive cells show different distributional patterns [30].

PP is a peptide hormone, which contains 36 amino acids, and is synthesized by F cells in the pancreatic islets [13]. The specific function of this peptide is not clear, however, it has been postulated that it is related to food intake inhibition [13] and Polak et al [24] reported that it promoted the secretion of gastric acid and stimulated the glycolysis of liver in avian species. It has been reported that PP-immunoreactive cells are conspicuously distributed in the peripheral regions of the pancreatic islets in mammalian species [3, 10, 18, 19, 28, 33, 34, 36]. In addition, the colocalization of these immunoreactive cells with serotonin-immunoreactive cells has been demonstrated in the pancreatic islets of the opossum [18] and cattle [21] though da Mota et al. [5] reported that PP-immunoreactive cells were not found in the pancreas of the three-toed sloth. In the present study, which is in agreement with previous studies [3, 10, 18, 19, 28, 33, 34, 36], PP-immunoreactive cells were detected in the outermost regions of the pancreatic islets, although rarely cells were intermingled with other immunoreactive cells in the mantle zone, where glucagon-immunoreactive cells predominated, followed by somatostatin-immunoreactive cells.

In conclusion, the regional distribution of endocrine cells in the pancreatic islets of Mongolian gerbil was found to be similar to that of other mammals, especially rodents, except for the topographically different distribution of somatostatin compared to that of other rodents. Cell core in the pancreatic islets of Mongolian gerbils were composed of centrally located insulin-immunoreactive cells and glucagon- and somatostatin-immunoreactive cells located in the mantle zone, PP-immunoreactive cells surrounded immunoreactive cells located in the mantle zone at the peripheral regions of pancreatic islets.

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