

An Immunohistochemical Study of the Insulin-, Glucagon-, Orexin A- and Ghrelin- Immunoreactive Cells in the Endocrine Pancreas of Guinea Pig

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Makale Kodu (Article Code): KVFD-2012-7220

Summary

The present study was conducted to clarify the regional distribution and relative frequency of endocrine cells secreting insulin, glucagon, orexin A (OXA) and ghrelin in the pancreatic islets of guinea pig, by specific immunohistochemical methods. In the pancreatic islets, most of insulin-IR cells were detected in the central region, partially in mantle region. Glucagon-IR cells were located in the periphery of the pancreatic islets. In addition, they had lower frequency in the central region. OXA and ghrelin- IR cells were identified in central and peripheral regions of the pancreatic islets. Clearly, OXA-IR cells exhibited a characteristic distribution pattern resemble to those for the glucagon- IR cells. However, ghrelin immunoreactivity exhibited a characteristic distribution pattern different from that of insulin-immunoreactive cells. In conclusion, some species-dependent unique distributions characteristics of endocrine cells in the pancreatic islets were observed in the guinea pig.

Keywords: Immunohistochemistry, Pancreas, Endocrine cell, Guinea pig

Kobay Endokrin Pankreasında İnsülin-, Glukagon-, Orexin A- ve Ghrelin- İmmunoreaktif Hücrelerin İmmunohistokimyasal Çalışması

Özet

Bu çalışma, kobay pankreatik adacıklarında immunohistokimyasal metodlar kullanarak insülin, glukagon, orexin A (OXA) ve ghrelin salgılayan endokrin hücrelerin bölgesel dağılımını ve nisbi frekansını ortaya koymak için yapıldı. İnsülin-IR hücrelerin çoğu pankreatik adacıkların merkez bölgesinde, kısmen de manto bölgesinde belirlendi. Glukagon-IR hücreler genellikle pankreatik adacıkların periferinde, çok az yoğunlukta ise merkez bölgede tespit edildi. OXA ve ghrelin-IR hücreler ise pankreatik adacıkların merkezi ve periferel bölgelerine lokalize oldu. Daha açık ifadeyle, OXA-IR hücrelerin lokalizasyonu glukagon-IR hücrelere benzerlik gösterdi. Buna karşın, ghrelin immunoreaktivitesi insülin-IR hücrelerinkinden farklı bir dağılım sergiledi. Sonuç olarak; kobayların pankreatik adacıklarındaki endokrin hücrelerin karakteristik dağılımı türe özgü farklılık göstermektedir.

Anahtar sözcükler: İmmunohistokimya, Pankreas, Endokrin hücre, Kobay

INTRODUCTION

The pancreas is formed by two distinct compartments, different in morphology and function: the exocrine portion, which secretes its digestive juice into the duodenum, and the endocrine portion, which produces hormones for the control of the carbohydrate metabolism¹. The endocrine pancreas, known as islets of langerhans, contains A-, B-, D- and F-cells producing glucagon, insulin, somatostatin and pancreatic polypeptide and probably also neuropeptide Y, respectively².

A few years ago, a fifth mammalian islet cell type, the ghrelin-producing epsilon cell, was described^{3,4}. However, the cell type that produces ghrelin in the pancreatic islets remains controversial, whether it be the A cells, B cells, the newly identified islet epsilon cells (ϵ -cell), or a unique novel islet cell type⁵⁻⁸. More recently, orexin A was also found in human and rodent pancreas where it is expressed only in a few endocrine cells, subsequently identified as B type insulin-



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IR cells by immunohistochemical techniques ⁹.

The pancreas is a valuable organ for endocrine studies, with the endocrine pancreas being extensively studied in diabetes ¹⁰. In addition, investigations of gastroenteropancreatic (GEP) endocrine cells are considered to be an important part of phylogenetic study ^{11,12}.

Many neuropeptides play a significant role in the regulation of both exocrine and endocrine pancreatic secretion in the rat ^{5,13}. Immunohistochemical localization of pancreatic hormones, several neuropeptides, has been determined in the pancreas of the rodents such as hamster ¹⁴, mouse ⁴, C57BL/6 mouse ¹⁵, ddN mouse ¹⁶, wood mouse ¹⁷, guinea pig ¹⁸, gerbil ¹⁹ and rat ^{5,20,21}.

It has been classically admitted that insulin immunoreactive cells are located in the central regions and glucagon immunoreactive cells are located in the peripheral regions ²². Also, orexin-A has been shown to localize to B cells ²³ and ghrelin has been shown to localize to B cells ⁶ and A cells ⁵, and to the newly discovered type of cell, that does not stain for any of the known islet hormones, and called the E cell ²⁴. But, many researches suggested that the regional distribution and relative frequency of immunoreactive (IR) endocrine cells in the pancreatic islets are different in different portions of the pancreas even within the same pancreas of the same animal ^{17,25} and a species-dependent characteristic distribution of pancreatic endocrine cells originating from feeding habits has also been suggested ²².

The initial focus of many studies was on insulin, somatostatin, glucagon, pancreatic polypeptide in the endocrine portion, and other neuropeptides found in the exocrine portion ^{16,26,27}. In the present study, the regional distribution and relative frequency of endocrine cells in guinea pig pancreas were examined by immunohistochemical method using specific antisera against insulin, glucagon, orexin A (OXA) and ghrelin.

MATERIAL and METHODS

Animals and Tissue Samples

Ten female guinea pig were used in this study. After the guinea pig were anaesthetized with pentathol (6 ml/kg), the left carotid artery was cannulated at the base of the neck

and allowed to exsanguinate. Tissue samples were taken from pancreas and fixed in 10% neutral-buffered formalin for 24 h. They were then dehydrated through graded ethanol and embedded in paraffin. Five μ m thick sections, mounted on poly-L-lysine coated glass slides, were obtained and processed for immunohistochemical staining.

Immunohistochemistry

Tissues were incubated in citrate buffer (10 mM citric acid, pH 6.0) for 20 min to retrieve antigenicity. Immunohistochemical staining was performed according to the manufacturer's protocol the SuperPicture™ 3rd Gen IHC Detection Kit (Invitrogen; Cat. No: 87-8973). Sections were incubated with primary antibodies for 16-20 h at 4°C. The working dilutions and the sources of the primer antibodies were listed in [Table 1](#). The primary antibodies were diluted in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin (BSA). Negative control sections were performed by replacing the primary antibodies with PBS. The sections were then incubated with HRP polymer conjugate 10 min at room temperature. Subsequently, the sections treated with DAB chromogen according to the kit instructions, washed in distilled water and counterstained with Mayer's hematoxylin. Finally, sections were dehydrated and coverslips mounted with mounting medium.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger ²⁸, including the replacement of specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen. Sections were examined with Olympus BX-51 microscope and photographs were taken.

RESULTS

Guinea pig pancreas was found to be consisted of exocrine and endocrine parts (pancreatic islets). The endocrine parts of the pancreas were scattered singly or in small groups of islets of various shapes and size in the interstitium of the exocrine parts.

The pancreatic islets of this study were distinguished as three distinct regions, central, mantle and peripheral regions, with their composition of immunoreactive (IR) cells. In this study, all four types of the IR endocrine cells were detected

Table 1. Details of antibodies used in this study

Tablo 1. Çalışmada kullanılan antikorlar

Primer Antibodies	Dilution	Trade Name	Cat. No.
Insulin	1:1000	Abcam	ab63820
Glucagon	1:200	Santa Cruz Biotechnology, Inc. Germany	sc-13091
Orexin A	1:1000	Chemicon (Millipore)	AB3704
Ghrelin	1:100	Santa Cruz Biotechnology, Inc. Germany	sc-50297

All antisera were raised in rabbit (polyclonal)

with the antisera against insulin, glucagon, orexin A and ghrelin in pancreatic islets of the guinea pig.

Insulin-IR Cells (B Cells): Round and/or oval shaped insulin-IR cells were abundant in the whole pancreatic islets. The most of insulin-IR cells were located in the central region of islets except for a small area. A few insulin-IR cells were detected in mantle region. Endocrine cells in islets were stained strongly with insulin (Fig. 1.A). Insulin positive cells were not found in the peripheral regions or in the exocrine portions.

Glucagon- IR Cells (A Cells): Glucagon-IR cells were located in the periphery of the pancreatic islets where the cell population is primarily A cells and a somewhat lower frequency of cells was noticed in the central region intermingled with insulin-IR cells. They were generally

shuttle or oval shaped. The intensity of glucagon staining was similar to insulin (Fig. 1.B). In addition, distribution of islets localized endocrine cells were more less than insulin. Glucagon-IR cells were not found in the exocrine portions or centroacinar cells.

Orexin A- IR Cells: Orexin A-IR cells were localized in central and peripheral regions of the pancreatic islets. The IR-cells were generally observed small clusters composed of 2-3 cells and they were in variable shapes. Orexin A-IR cells exhibited a characteristic distribution pattern resemble to those for the glucagon- IR cells. However, the intensity of the staining was not so strong as in glucagon-IR cells (Fig. 1.C). Orexin A-IR cells were not detected in exocrine portion or centroacinar cells.

Ghrelin- IR Cells: Ghrelin-IR cells were usually round or

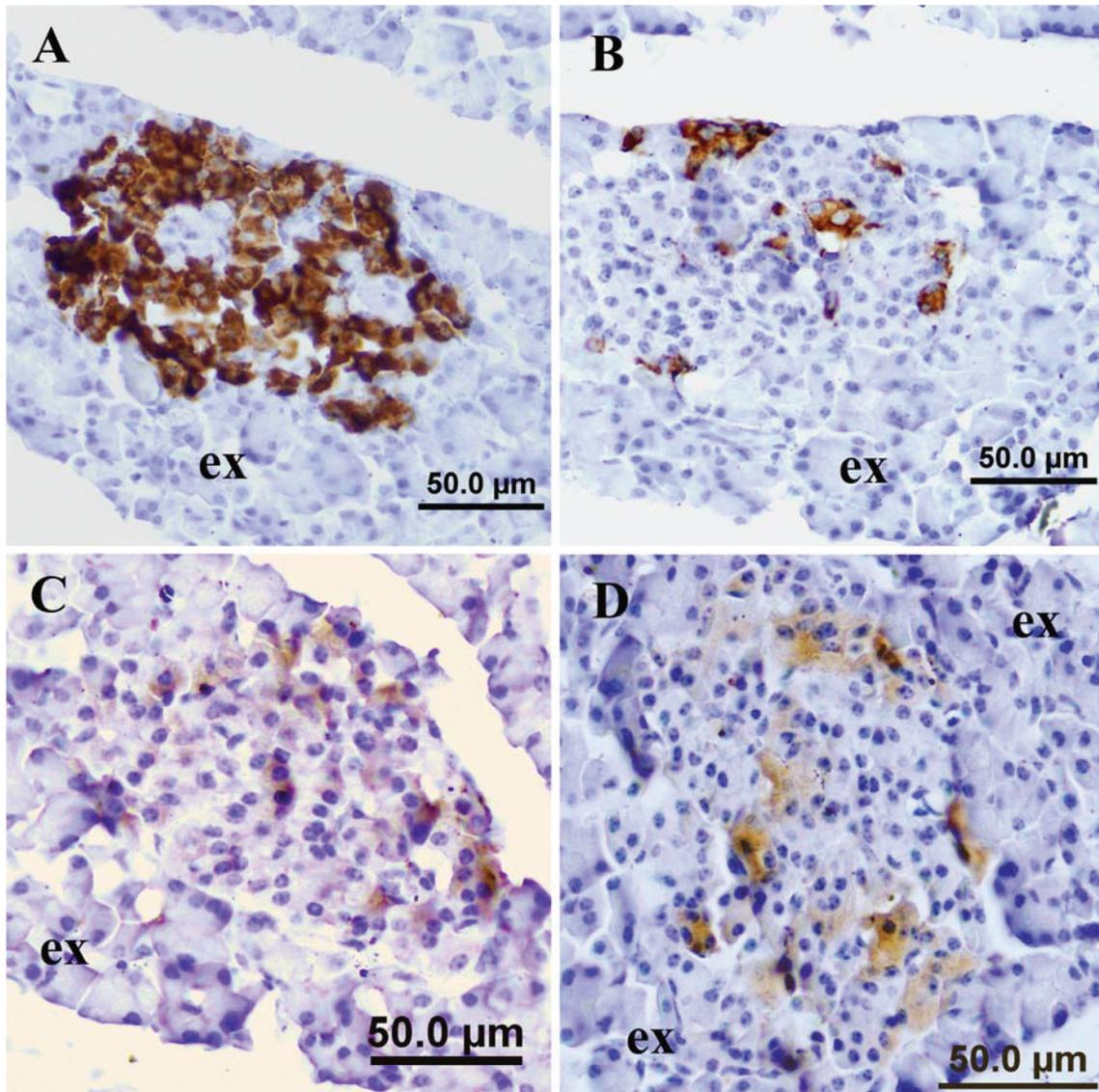


Fig 1. Guinea pig pancreatic islets showing immunoreactive cells (ex: exocrine), A- Insulin-IR cells, B- Glucagon-IR cells, C- Orexin A-IR cells, D- Ghrelin-IR cells

Şekil 1. Kobay pankreatik adacıklarında immunoreaktif hücrelerin görünümü (ex: ekzokrin), A- İnsulin-IR hücreler, B- Glukagon-IR hücreler, C- Oreksin A-IR hücreler, D- Ghrelin-IR hücreler

ovoid in shape and were located in both the peripheral and central regions of the islets, either as single cells or small clusters of cells. However these IR cells were more diffuse in peripheral regions of some islets. Cells positive for ghrelin immunoreactivity exhibited a characteristic distribution pattern different from that of insulin-immunoreactive cells. The intensity of ghrelin staining in endocrine cells were more weak than that of insulin- and glucagon-IR (Fig. 1.D). Ghrelin-IR cells were not detected in exocrine portion or centroacinar cells.

DISCUSSION

The function of hormones released from pancreatic endocrine cells is directly related to the regulation of pancreatic digestive enzymes and serum glucose levels²⁹. Therefore the different distribution patterns and frequency of these pancreatic endocrine cells are considered to be a result of differences in feeding habits, especially for glucose and proteins. This study was carried to determine the existence and distribution of pancreatic endocrine cells in guinea pig pancreas.

Insulin is synthesized in the B cells of the pancreatic islets and regulates the blood glucose levels³⁰. In the mammals, the regional distribution and the relative frequency of pancreatic insulin-IR cells were reported in the hamster¹⁴, rat²⁷, guinea pig¹⁸, C57BL/6 mouse¹⁵, gerbil¹⁹, wood mouse¹⁷, and various laboratory animals²². According to the previous studies, insulin immunoreactive cells were observed in the central regions of the pancreatic islets and other cells, such as glucagon- and somatostatin-IR cells, surrounded them. However, it was described that insulin-IR B cells occupied the majority of the periphery regions of islets in monkey and possum pancreas^{31,32}. In the present study, most of the insulin-IR cells were restricted to the central and mantle regions of pancreatic islets in the guinea pig. The results of this study were compatible with the findings reported by previous studies on rodents except for monkey and possum^{14,15,18,19,22,27}.

Glucagon is synthesized in the A cells of the pancreas and also participates to the regulation of blood glucose concentrations³⁰. The distribution of glucagon-IR cells was quite different from species regardless of the same mouse strains. Although most of the glucagon-IR cells were situated in the peripheral regions of the pancreatic islets, they were also demonstrated in the central regions in the both splenic and duodenal lobes of the ddN mouse¹⁶. In the other examinations performed on mouse strains^{25,33}, glucagon-IR cells were mainly detected to peripheral regions but some these cells were also present in the central regions of the pancreatic islets. However, Ku et al.¹⁵ reported that these immunoreactive cells were mantle and peripheral located in C57BL/6 mouse. Interestingly, in the monkey, glucagon-IR cells were found in the central regions of pancreatic islets where insulin-IR cells were numerous located in most

of the domestic animals³². However, some researchers reported that glucagon-IR cells were found in peripheral regions of rat pancreatic islets^{27,32}. In the present study, glucagon-IR cells were located in the peripheral regions of pancreatic islets; a somewhat smaller number of these cells were also observed in the central regions intermingled with insulin-IR cells. These results were found to be similar to that of mouse strains^{16,25,33} but different from the other species^{27,32}.

The orexin family is a complex system composed of two neuropeptides, orexin-A and orexin-B (OXA and OXB), and two cognate receptors, orexin type 1 and orexin type 2 (OX1R and OX2R). Recently, orexins have been studied extensively to comprehend their complex functional roles. In fact, since the first identification of OXA and OXB in rat hypothalamus³⁴, the presence of this system has been found in both the central and peripheral nervous systems as well as in several other different tissues and organs³⁵. Recently, it has been found in peripheral tissues and, particularly, in endocrine cells and neurons of the enteric nervous system (ENS) of different portions of the gastrointestinal tract and in the pancreas and salivary glands^{9,36}.

In the rat, guinea-pig and mice pancreas, OXA immunoreactivity was detected in B cells of pancreatic islets. Furthermore, insulin-IR islet cells also displayed OX1R-like immunoreactivity, and OX1R mRNA was detected in the rat pancreas³⁷. In double immunostaining in the human pancreas, a great majority of insulin-IR cells was simultaneously positive for orexin-A³⁸. Conspicuously, OXA-immunoreactivity was detected in almost 60% of insulin positive cells in pancreatic islets of domestic animals⁹. In contrast, OXA and OX1 immunoreactivity were found in glucagon-IR cells in rat pancreatic islets²⁰. Interestingly, OX1R-positive cells were observed in the periphery of pancreatic islets of normal and in both the peripheral and central regions of the islet of diabetic rats^{39,40}. Similar to the findings reported for rats, OXA-IR cells were found peripherally and more less centrally located in pancreatic islets in the present study.

Ghrelin, a 28-amino acid peptide which was isolated from rat and human stomach, acts as an endogenous ligand for GHS-R⁴¹. Pancreas ghrelin is emerging as a key player in the regulation of insulin secretion by the B cell, suggesting that it may play an important role in glucose metabolism^{42,43}.

Recently, ghrelin was identified to be present in pancreatic islet cells by immunostaining and immunofluorescence methods. Immunostaining for ghrelin was observed in B-cells of human pancreatic islets⁶; however, it was detected in the A-cells of rat and human pancreas⁵. Furthermore, ghrelin-IR cells correspond to glucagon-IR cells, and GHS-R-like (ghrelin receptor) immunoreactivities were located in glucagon- and insulin-IR cells of the rat pancreas^{21,44}. In a study performed on rats, were detected ghrelin immunostaining either independently of glucagon staining or together with glucagon immunostaining in a minority of A-cells while not detect ghrelin immunostaining in B-cells²⁴.

Interestingly, in the examinations performed on rodent, ghrelin was observed in a newly discovered type of cell, that does not stain for any of the known islet hormones, and called the epsilon cell (ϵ -cell)^{4,8,45}. Thereafter, the presence of these cells was confirmed in fetal and adult human pancreas. Epsilon cells were usually round or ovoid in shape and were often located at the periphery of the islets, either as single cells or small clusters of cells⁴⁶. In the present study, ghrelin-IR cells were detected peripheral and central regions of the islets. Conspicuously, it was demonstrated that significantly less B-cell area (central region) and markedly larger A- cell areas (peripheral region) of islets. The distributions of these cells were resemble with the results of rats^{21,44} but different from mammalian^{4,8,24,45,46}. These differences might be due to different antisera, methods and/or species used in each study.

The present study was revealed the existence and distribution of endocrine cells in the pancreas. In this study; insulin, glucagon, OXA and ghrelin were detected to exist in the pancreatic islets of the guinea pig. The general distribution patterns of pancreatic endocrine cells of the guinea pig was similar to those of some rodent and other species. However, some species-dependent unique distributions characteristics of endocrine cells in the pancreatic islets were also observed in the present study. The characteristic existence may be variety in feeding habits (between an animal species) and/or caused by this species specific differences among species.

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