*Kafkas Univ Vet Fak Derg*
*15 (4): 611-614, 2009*

**RESEARCH ARTICLE**

# Immunohistochemical Distribution of Insulin-, Glucagon-, and Somatostatin-Containing Cells in the Pancreas of the Rat *(Wistar albino)*

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**Makale Kodu (Article Code): 2009/086-A**

## Summary

The aim of the present study was to determine the regional distribution of the insulin-, glucagon-, and somatostatin-containing cells in the pancreas of rat. Tissue samples were obtained under deep ethyl ether anaesthesia from 10 adult rat *(Wistar albino)*. Tissue sections were stained with Crossmon's connective tissue staining for general observations. Sections were further processed for standart immunohistochemical techniques using the avidin- biotin-complex method for the distribution of insulin-, glucagon-, and somatostatin-containing cells. It was monitored that insulin-containing B cells were located central regions of the islets of Langerhans whereas glucagon- containing A cells and somatostatin- containing D cells were located peripheral regions of the islets. Somatostatin- containing cells were rare in the islets of Langerhans. Our findings make additional contribution to those of pancreatic studies of rats which have been often used as an animal model in the studies dealing with diabetes or islet cell tumours.

**Keywords:** *Insulin, Glucagon, Somatostatin, Pancreas, Rat*

# Rat *(Wistar albino)* Pankreasında İnsulin-, Glukagon- ve Somatostatin-İçeren Hücrelerin İmmunohistokimyasal Olarak Dağılımı

## Özet

Bu çalışmanın amacı insulin-, glukagon-, ve somatostatin-içeren hücrelerin rat pankreasında bölgesel olarak dağılımının belirlenmesidir. Doku örnekleri, derin etil anestezisi altındaki 10 adet erişkin rattan alındı. Doku kesitleri genel görünüm için Crossmon'ın bağ doku boyasıyla boyandı. Kesitlere insulin-, glukagon-, ve somatostatin içeren hücrelerin dağılımının belirlenmesi için standart Avidin-biotin complex (ABC) metodu uygulandı. İnsulin içeren B hücrelerinin adacıkların merkezinde yerleştiği görüldü. Glukagon-içeren A hücreleri ve somatostatin-içeren D hücreleri adacıkların periferinde yerleşmişti. Somatostatin-içeren hücreler Langerhans adacıklarında az sayıdaydı. Sonuç olarak elde edilen bulgular sıklıkla diabet ya da adacık hücre tümörleri gibi hastalıklarda hayvan modeli olarak kullanılan ratların pankreasında yapılan çalışmalara katkıda bulunacaktır.

**Anahtar sözcükler:** *İnsulin, Glukagon, Somatostatin, Pankreas, Rat*

## INTRODUCTION

Pancreas in the vertebrates is subdivided into two parts: One of them is exocrine part where digestive enzymes are released and the other one is endocrine part where regulatory hormones are released into blood circulation [1]. The endocrine part, formed by the islets of Langerhans, is multihormonal unit composed of at least four types of cells; the insulin (B cells), the glucagon (A cells), the somatostatin (D cells), and the pancreatic polypeptide (PP cells) [2].

Insulin is synthesized in the B cells of the pancreatic islets and regulates the blood glucose levels. Glucagon

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is synthesized in the A cells of the pancreas and regulates blood glucose levels as well [3]. Somatostatin is a polypeptid hormone [4] which is also known as a somatotropin release-inhibiting factor (SRIF) and secreted by pancreatic δ (D) cells [5,6]. Somatostatin is known to be a multifunctional hormone that inhibits the secretion of a large number of hormones including insulin, glucagon, gastrin and cholecystokinin [4].

The purpose of this study was to determine the regional distribution of the insulin-, glucagon-, and somatostatin-containing cells in the pancreas of the rat by immunohistochemistry using specific antisera against insulin, glucagon, and somatostatin.

## MATERIAL and METHODS

In the present study, ten adult rats *(Wistar albino)* were used without any gender distinction. Tissue specimens were dissected and obtained under deep ethyl ether anaesthesia from the lobes of the pancreas. Samples from the pancreas were fixed in Bouin's solution for 12 h at 4ºC. After paraffin embedding, serial tissue sections were cut at 5-6 µm in thickness. Sections of each tissue were stained with Crossmon's connective tissue stain [7] for general observations.

### *Immunohistochemistry*

Each section was deparaffinized, rehydrated and then immunostained with the avidin-biotin-complex (ABC) method [8]. The endogeneous peroxidase and non-specific binding sites for andibodies were supressed by treating sections with 0.5% hydrogen peroxide for 30 min and 10% normal rabbit serum for 10 min at room temperature, respectively. Furthermore, sections were processed for standart immunohistochemical techniques. The working dilutions and the sources of antibodies used are listed in the *Table 1*. Peptide specific antibodies isolated from mammalian species were used. Negative controls were carried out by incubating sections with phosphate-buffered saline (PBS) instead of the primer antiserum. Negative controls were also conducted with tissue sections from the gastrointestinal tract of rabbits known to contain the hormones studied. The sections were incubated in primary antisera in PBS-containing bovine serum albumin (2.5%) and Triton X-100 (0.2%) for 1 h at room temperature. Subsequently, the binding of primary antisera was detected using rabbit-antimouse antisera and Strept ABC. Finally, the chromogen protocol was used the reveal the distribution of bound peroxidase activity [9].

***Table 1.*** *The primary and secodary antibodies and their dilutions*
***Tablo 1.*** *Primer ve sekonder antikorlar ve dilusyonları*

| Antisera | Working Dilutions | Sources |
|---|---|---|
| Insulin | 1: 40 | Signet |
| Glucagon | 1: 40 | Signet |
| Somatostatin | 1: 100 | Dako |
| Goat-anti-rabbit Ig G | 1: 100 | Zymed |
| Rabbit anti-mouse Ig G | 1: 100 | Dako |
| Strept ABC | 1: 50 | Dako |

## RESULTS

Rat pancreas was found (as expected) to be consisted of exocrine and endocrine (islets of Langerhans) parts. 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 islets of Langerhans were composed of two different regions, which were central and peripheral regions. Positive immunohistochemical reactions were observed in the pancreatic islets of rat against insulin-, glucagon-, and somatostatin containing cells. No specific staining was found in the exocrine part of pancreas.

### *Insulin-containing cells (B cells)*

Insulin-containing cells were abundant in the whole islets of Langerhans. Those cells were located in the central regions of the islets. B cells were found to have round shape *(Fig 1)*.

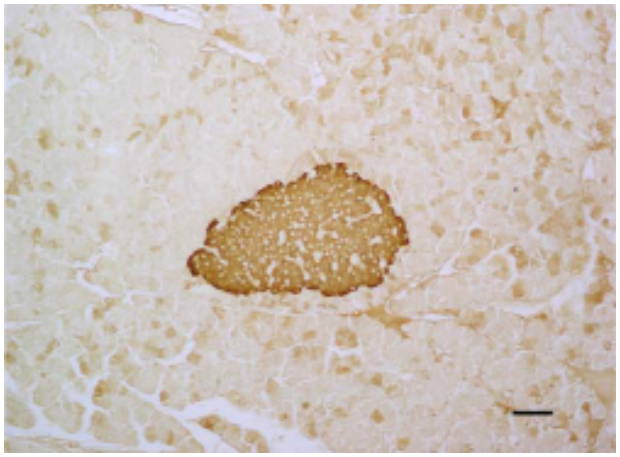

**Fig 1.** Insulin-containig cells (B cells) in islets of Langerhans. Bar: 100 µm
**Şekil 1.** Langerhans adacıklarında insulin-içeren hücreler (B hücreleri). Bar: 100 µm

### *Glucagon-containing cells (A cells)*

Glucagon-containing cells were located in peripheral regions of the islets. They were surrounded by the B cells and had round shape *(Fig 2)*.

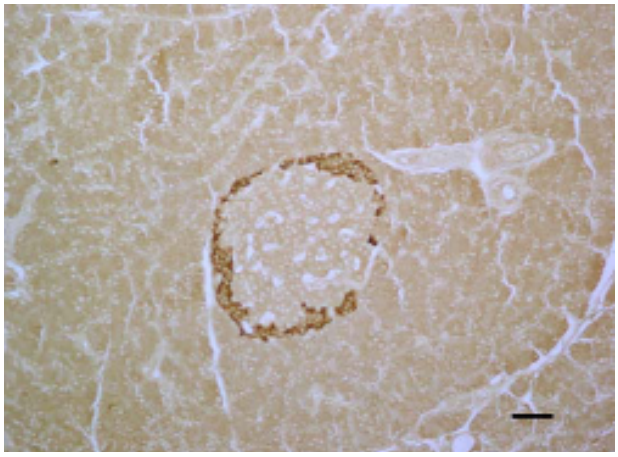

**Fig 2.** Glucagon-containing cells (A cells) in islets of Langerhans. Bar: 100 µm

**Şekil 2.** Langerhans adacıklarında glukagon-içeren hücreler (A hücreleri). Bar: 100 µm

***Somatostatin-containing cells (D cells)***

Somatostatin-containing cells were found rarely in the islets of Langerhans. D cells were localised especially in the peripheral regions of the islets. They were found to have irregular shape *(Fig 3)*.

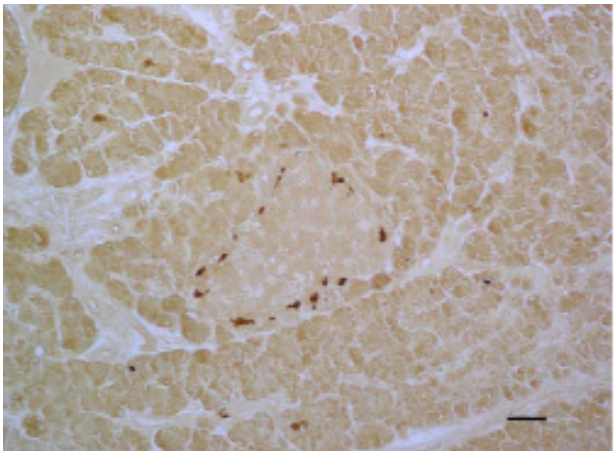

**Fig 3.** Somatostatin-containig cells (D cells) in islets of Langerhans. Bar: 100 µm

**Şekil 3.** Langerhans adacıklarında somatostatin-içeren hücreler (D hücreleri). Bar: 100 µm

## DISCUSSION

The pancreas is composed of two secretory parts, which are the insular or endocrine part and the acinar or the exocrine part [2]. In this study, pancreas was found to be consisted of exocrine and endocrine (islets of Langerhans) parts, as explained in literature. It was reported that insulin-immunoreactive cells were arranged in the central region of pancreas islets in wood mouse [10] and hamster [11]. However, it was described that insulin-immunoreactive B cells occupied the majority of the periphery regions of islets in monkey pancreas [12]. In the present study, insulin-containing cells were observed in the central regions of islets of Langerhans, as in agreement with previous studies [10,11,13].

In monkey [12] and equine [14], glucagon-immunoreactive cells were found in the central regions of pancreatic islets where insulin-immunoreactive cells were numerously located in most of the domestic animals. Glucagon-containing cells were seen peripheral regions of pancreatic islets in our study. Sujatha et al.[12] and Helmstaedter et al.[14] reported that somatostatin-immunoreactive cells were found mostly with glucagon- immunoreactive cells in the center of pancreatic islets. However, somatostatin-containing cells were located peripheral regions of the islets of Langerhans in the current study as described in some of the studies [10,11,13]. Taken together, cellular pattern of islets has a species difference that may eventually give rise to functional significance.

There were several immunohistochemical and morphometric studies on the glucagon-, insulin-, and somatostatin-immunoreactive cells in the pancreas [15-21]. It has been indicated that in diabetic state, pancreatic beta-cells showed a weak immunostaining for insulin, appears to suggest that induction of regenerative stimulus in diabetic state triggers pancreatic regenerative processes, thereby restoring functional activities of the pancreas [15]. It was demonstrated that significantly less B-cell area and markedly larger A- and D- cell areas within the diabetic islets were found when compared with the non-diabetic and pre-diabetic islets [19]. In another study, they have observed a direct relationship between total hormone area and hormone content, in islets from normal and hyperglycemic diabetic dogs [20]. This findings suggest that there are acute effects of insulin or of normalization of glycemia on the A- and D-cells, to reduce hormone content in diabetes. Badawoud [21] demonstrated that the B cell volume density was significantly lower in the diabetic group, while islet volume density, islet diameter, islet volume and absolute islet cell numbers were significantly greater in the diabetic group. The B cell nuclear diameter and volume were not significantly different in the diabetic group. This study indicated that maternal diabetes induced foetal islet hypertrophy and caused an increase in the total islet cell number [21].

In conclusion, it was monitored that insulin-containing B cells were located central regions of the islets whereas glucagon- containing A cells and somatostatin- containing D cells were located peripheral regions of the islets. Somatostatin- containing cells were rare in the islets of Langerhans. Therefore, the findings make additional contribution to those of pancreatic studies of rats which have been often used as an animal model in the studies dealing with diabetes or islet cell tumours.

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