Kafkas Üniv Vet Fak Derg
13 (2): 133-137, 2007
DOI:10.9775/kvfd.2007.19-A

Immunohistochemical Localization of Serotonin-, Gastrin-, and Somatostatin-immunoreactive Endocrine Cells in the Duodenum of the Rat (Wistor albino)

Ebru KARADAĞ SARI* Mumtaz NAZLI* Hakan KOCAMIŞ*
Nurhayat GÜLMEZ* Şahin ASLAN* Turgay DEPREM*

Kafkas Üniversitesi Veteriner Fakültesi Histoloji - Embriyoloji Anabilim Dalı, Kars – TÜRKİYE

Yayın Kodu: 2007/19-A

Summary

Most of the gastrointestinal hormones are produced in the small intestine particularly in the duodenal mucosa. The objective of the current study was to investigate the distribution of the serotonin-, gastrin-, and somatostatin-immunoreactive endocrine cells in the duodenum of rat by immunohistochemical techniques using antisera against serotonin, gastrin, and somatostatin. Numerous serotonin-immunoreactive cells, and very few gastrin-, and somatostatin-immunorective cells were seen in the rat duodenum.

Keywords: *Immunohistochemistry, Duodenum, Rat*

Serotonin-, Gastrin-, ve Somatostatin-imunoreaktif Endocrin Hücrelerin Rat (Wistor albino) Duodenumunda İmmunohistokimyasal Lokalizasyonu

Özet

Gastrointestinal hormonların bir çoğu ince barsaklarda, özellikle de duodenum mukozasında üretilir. Bu çalışmanın amacı, rat duodenumunda serotonin, gastrin ve somatostatine karşı antikorlar kullanarak immunohistokimyasal yöntemlerle serotonin-, gastrin-, ve somatostatin-immunoreaktif hücrelerin dağılımının incelenmesidir. Rat duodenumunda çok sayıda serotonin-immunoreaktif hücre ile çok az sayıda gastrin-, ve somatostatin-immunoreaktif hücre görüldü.

Anahtar sözcükler: *İmmunohistokimya, Duodenum, Rat*

İletişim (Correspondence)
Phone: +90 474 2426800/1340
e-mail: ekaradag84@hotmail.com

INTRODUCTION

The gastrointestinal tract is lined with a monolayer of cells that undergo parpetual and rapid renewal. Four principal, terminally differentiated cell types populate the monolayer, enterocytes, goblet cells, Paneth cells, and enteroendocrine cells [1]. Gastrointestinal endocrine cells dispersed through the epithelia and gastric glands of the alimantery tract synthesize various kinds of gastrointestinal hormones [2,3]. Most of these hormones are produced in the small intestine particularly in the duodenal mucosa [4] . At least 10 different endocrine cell types have been described in the small intestine of mammals [5]. Gastrointestinal hormones as regulatory peptides that appear to be major components of bodily integration and have important regulatory actions on physiological function of the gastrointestinal tract [6].

The recent developments of immunohistochemical procedures and the specific antibodies raised against peptides have led researchers to revealing the existence of different endocrine cells in the intestine, each of which synthesises and stores a distinct peptide hormone or biogenic amine.

The objective of the current study was to investigate the distribution of the serotonin-, gastrin-, and somatostatin-immunoreactive endocrine cells in the duodenum of rat by immunohistochemistry using antisera against serotonin, gastrin, and somatostatin.

MATERIALS and METHODS

In this study, ten adult rats were used without any sexual distinction. Tissue specimens were dissected under deep ethyl ether anaesthesia from the duodenum of these rats. Samples from the duodenum were fixed Bouin's solution . After parafin embedding, 5-6 μ serial sections were prepared.

Each section was deparaffinized, rehydrated and immunostained with the avidin-biotin-complex (ABC) method [7] . 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. Negative controls were carried out by incubating sections with phosphate-buffered saline (PBS) instead of the primer antiserum. Positive 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 antsera in PBS-containing bovine serum albumin (2.5%) and Triton X-100 (0.2%) for 1 hour at room temperature. Subsequently, the binding of primary antisera was detected using rabbit-antimouse antisera and Strept ABC. Finally the chromogen protocal was used the reveal the distribution of bound peroxidase [8] .

Table 1. The primary antibodies and their dilutions
Tablo 1. Primer antikor ve dilusyonları

Antisera	Working Dilutions	Sources
Serotonin	1: 40	Signet
Gastrin	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

Immunohistochemical procedures led to the identification of endocrine cells immunoreactive to serotonin, gastrin and somatostatin in the rat duodenum. Serotonin-, gastrin-, and somatostatin-immunoreactive cells were observed in the duodenum of the rat. Some of these endocrine cells had apical cytoplasmic processes that extended to the glandular or intestinal lumen. The frequency of these immunoreactive cells in the duodenum are shown in the *Table 2*.

Serotonin-immunoreactive endocrine cells were monitored localazing over the crypts and the villi epithelium. These cells were triangular, irregular or bipolar in shape. Serotonin-immunoreactive cells were highest freguency in the duodenum *(Figure 1)*.

Table 2. The frequency of the endocrine cells in the duodenal mucosa of the rats
Tablo 2. Ratların duodenum mukozasındaki endokrin hücrelerin sıklığı

Antisera	Duodenal mucosa
Serotonin	++
Gastrin	+
Somatostatin	+

The frequency: '+': 1-10 immunoreactive cells , '++': 10-20 immunoreactive cells.

Gastrin-immunoreactive endocrine cells were situated in the duodenal mucosa at very low frequencies. These immunoreactive cells were bipolar or triangular in shape *(Figure 2)*.

Only very few somatostatin-immunoreactive endocrine cells were located in the duodenal mucosa. They were particularly localized in the duodenal glands. These immunoreactive cells were irregular or bipolar in shape *(Figure 3)*.

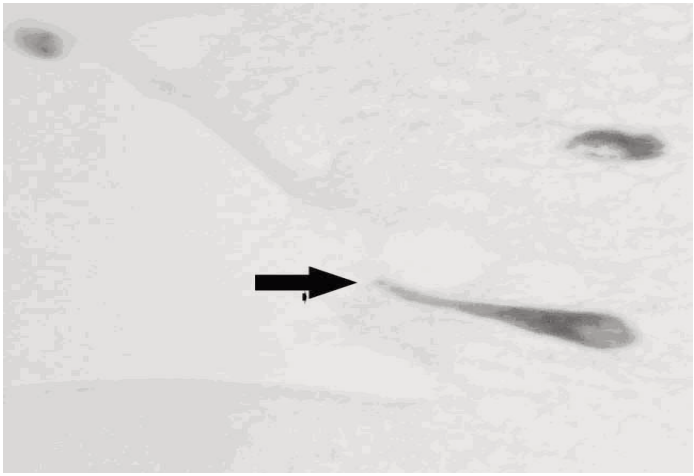

Fig 1: Serotonin-immunoreactive cells in the duodenum mucosa. ABC. Bar: 50 mm

Şekil 1: Doudenum mukozasında serotonin-immunoreaktif hücreler. ABC. Bar: 50 mm

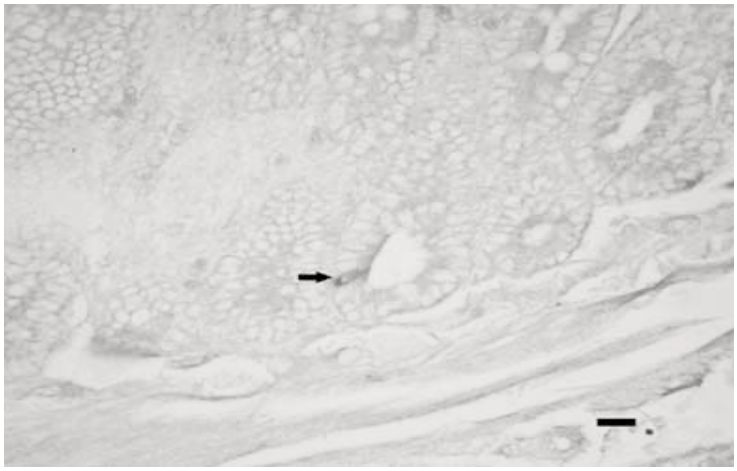

Fig 2: Gastrin-immunoreactive cell in the duodenum mucosa. ABC. Bar: 50 µm

Şekil 2: Doudenum mukozasında gastrin-immunoreaktif hücreler. ABC. Bar: 50 µm

Fig 3: Somatostatin-immunoreactive cell in the duodenum mucosa. ABC. Bar: 50 µm

Şekil 3: Duodenum mukozasında somatostatin-immunoreaktif hücreler. ABC. Bar: 50 µm

DISCUSSION

In the current study serotonin-, gastrin-, and somatostatin-immunoreactive cells were observed in the duodenum of the rat.

Serotonin consists of monoamins and widely distributed in the nervous system and gastro-entero-pancreatic endocrine cells [9]. The main function of serotonin are the inhibition of gastric acid secretion and smooth muscle contraction in the gastrointestinal tract [10] . It has been reported that serotonin-immunoreactive cells are a moderate number in philippine carabao [11], low number in wild boar [12] duodenum. It has also been reported that numerous serotonin-immunoreactive cells are distributed over the epithelium of both crypts and villi in the ruminant duodenum [13] . The results of our study are in parallel with Mimoda et al.[13] findings.

Gastrin is heptadecapeptide with 17 amino acids [14]. Gastrin is mostly distributed in the mucosa of gastric antrum, the mucosa of the jejenum and the central nervous system. Gastrin has a wide range of biological actions [15] . The most potent actions of gastrin are stimulation of gastric acid secretion [16]. Our findings are general agreement with the data reported in most of the avian[17-19] and mammalian[11-13,20] species in terms of gastrin-immunoreactive cells dis-tribution. However it has been reported that gastrin-immunoreactive cell was not found in the duodenal mucosa of the freshwater turtle [21]

Somatostatin was isolated from the hypothalamus of sheep for the first time [22]. Somatostatin is produced by endocrine-like (D cells) of the gut and pancreas and peripheral nevre cells. Somatostatin appears to inhibit the secretion of the many gastrointestinal hormones and may be an important regulator for gastrointestinal functions [23]. It has been described that somatostatin-containing cells could occasionally be found in the submucosal glands of the duodenum [24]. In this study somatostatin-immunoreactive cells were seen especially in the duodenal glands of the rat duodenum. The heterogeneity and concentration of endocrine cells in the duodenum may be related to the regulation of the secretion of the pancreatic juice and bile as well as the control of the function of the stomach and the small intestine [25,26]. In conclusion serotonin-, gastrin-, and somatostatin-immunoreactive cells were monitored in the duodenum mucosa in the rat.

REFERENCES

1. **Roth KA, Hertz JM, Gordon JI:** Mapping enteroendocrine cell populations in transgenic mice reveals unexpected degree of complexity in cellular differentiation within the gastrointestinal tract. *J Cell Biol*, 110 (5): 1791-1801, 1990.
2. **Ohara N, Kitamura N, Yamada J, Yamashita T:** Immunohistochemical study of gastro-entero-pancreatic endocrine cells of the herbivorous Japanese field vole. Microtus montebelli. *Res Vet Sci,* 41, 21-27, 1986.
3. **Bell FR:** The relevance of the new knowledge of gastrointestinal hormones to veterinary science. *Vet Sci Commun,* 2, 305-314, 1979.
4. **Tanyolaç A:** Özel Histoloji. s. 44. Yorum Basın Yayın San. Ltd. Şti. Ankara, 1999.
5. **Solcia E, Capella C, Fiocca R, Sessa F, LaRosa S, Rindi G:** Disorders of the Endocrine System. in Pathology of the Gastrointestinal Tract (ed. S. C. M. a H. Goldman), pp. 295-322. Williams and Wilkins, Philadelphia, 1998.
6. **Yao YL, Xu B, Zhang WD, Song YG:** Gastrin somatostatin and experimental disturbance of the gastrointestinal tract in rats. *World J Gastroentero,* 7 (3): 399-402, 2001.
7. **Hsu SM, Raine L, Fanger H:** Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) prosedures. *J Histochem Cytochem,* 29, 577-580, 1981.
8. **Shu S, Ju G, Fan L:** The glucose oxidase-DAB-nickel in peroxidase histochemistry of the nervous system. *Neurosci Lett,* 85, 169-171, 1988.
9. **El-Salhy M, Winder E, Lundqvist M:** Comparative study of serotonin-like immunoreactive cells in the digestive tract of vertebrates. *Biomed Res,* 6, 371-375, 1985.
10. **Guyton AC:** Secretory Functions of the Alimentary Tract. **In,** Guyton AC (Ed): Textbook of Medical Physiology. 8th ed, pp. 801-815. WB Sounders Philedelphia, 1988.
11. **Baltazar ET, Kitamura N, Hondo E, Yamada J, Maala CP, Simborio LT:** Immunohistochemical study of endocrine cells in the gastrointestinal tract of the philippine carabao (Bubalus bubalis). *Anat Histol Embryol,* 27, 407-411, 1998.
12. **Dall' Aglio C, Scocco P, Ceccarelli P, Pedini V:** Neuroendocrine cells in the gastrointestinal tract of wild boar. *Anat Histol Embryol,* 27, 381-385, 1998.
13. **Mimoda T, Kitamura N, Hondo E, Yamada J:** Immunohistochemical colocalization of serotonin, substance P and met-enkaphalin-arg-gly-leu in the endocrine cells of the ruminant duodenum. *Anat Histol Embryol,* 27, 65-69, 1998.
14. **Rooney PJ, Grennan D, Miller J:** Gastrin: A review. *Current Med Res Opin,* 2 (5): 295-304, 1974.

15. **Ryberg B, Tielemans Y, Axelson J, Carlsson E, Hakanson R, Mattsson H, Sundler F, Willems G:** Gastrin stimulates the self-replication rate of enterochrommoffinlike cells in the rat stomach: Effects of omepazole, ranitidine, and gastrin-17 in intact and antrectomized rats. *Gastroentero,* 99, 935-942, 1990.
16. **Waldum HL, Sandvic AK, Brenna E, Petersen H:** Gastrin-histamin sequence in the regulation of gastric acid secretion. *Gut,* 32, 698-701, 1991.
17. **Gülmez N, Nazli M, Aslan Ş, Liman N:** Immunolocalization of serotonin, gastrin, somatostatin, and glucagon in entero-endocrine cells of the goose (Anser anser). *Acta Vet Hung,* 51 (4): 439-449, 2003.
18. **Polak JM, Paersa AGE, Adams C, Garand JC:** İmmunohistochemical and ultrastructural studies on the endocrine polypeptide (APUD) cells of the avian gastrointestinal tract. *Experientia,* 30 (5): 564-567, 1974.
19. **Salvi E, Renda T:** Immunohistochemical studies on the ontogenesis of some endocrine cells in the chicken antrum and duodenum. *Basic Appl Histochem,* 30, 307-316, 1986.
20. **Capella C, Solcia E:** The endocrine cells of the pig gastrointestinal mucosa and pancreas. *Arch Histol Jap,* 35, 1-29, 1972.
21. **Gencer Tarakcı B, Simsek Koprucu S, Yaman M:** An immunohistochemical study on the endocrine cells in the gastrointestinal tract of the freshwater turtle, Mauremys caspica caspica. *Turk J Vet Anim Sci,* 29, 581-587, 2005.
22. **Park J, Chiba T, Yamada T:** Mechanisms for direct inhibition of canine gastric parietal cells by somatostatin. *J Biol Chem,* 262, 14190-14196, 1987.
23. **Yao YL, Song YG, Zhang WD:** Variations of G cells and D cells in experimental spleen asthenia and its significance. *Zhonng Zhongx Jie Piew Zaz,* 7, 8-11, 1999.
24. **Ceccarelli P, Gargiula AM, Pedini M:** The endocrine cells in the gastro-enteric tract of adult fallow deer (Dama dama L.). *Anat Histol Embryol,* 24, 171-174, 1995.
25. **Kitamura N, Yamada J, Yamashita T, Yanaihara N:** Endocrine cells in the gastrointestinal tract of the cat. *Biomed Res,* 3, 612-622, 1982.
26. **Krause WJ, Yamada J, Cutts H:** Quantative distribution of enteroendocrine cells in the gastrointestinal tract of the adult opposum, Didelphis virginia, *J Anat,* 140, 591-605, 1985.