Regional and mucosal distributions of some intestinal immunoreactive endocrine cells in New Zealand white rabbit

(Orzyctolagus cuniculus L.)

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Summary

The aim of this study was to detect the regional and mucosal distribution of endocrine cells that secrete glucagon, somatostatin, Chyhololecystokin-8 (CCK-8), serotonin, secretin, substance P (SP) and histamine in the small and large intestine of New Zealand white rabbit (Orzyctolagus cuniculus L.) using immunohistochemical peroxidase-antiperoxidase (PAP) method. It was found that most of the immunoreactive (IR) endocrine cells, which are oval- or spindle-shaped, are spotted in the basal parts of the relevant glands. It was noticed that cells in the lamina epithelialis of small and large intestine is linked to the lumen and that the cells in their glands cannot reach the lumen. Immunoreactive cells for glucagon, somatostatin, serotonin, secretin and SP were identified in lamina epithelialis of the small and large intestine. It was seen that secretin, SP and histamine-IR cells are rarely deployed throughout the intestinal tract. It was determined that somatostatin-IR cells were identified throughout the intestinal tract. In conclusion, the immunohistochemical study shows that gastrointestinal tract of this species contained different types of endocrine cells similar to those found in other vertebrate species. However, some species-dependent unique distributions and frequencies of endocrine cells were also observed in the present study.

Key words: Endocrine cell, Immunohistochemistry, Intestine, Rabbit

Introduction

Gastrointestinal endocrine cells are spread along the epithelia and glands of the gastrointestinal tract. This cells produce diverse hormones and have regulatory roles in the physiological functioning of the digestive tract (Bell, 1979). Glucagon is secreted from pancreatic A-cells (Holst, 2000) and is responsible for reducing insulin secretion in the digestive tract and inhibiting digestive secretion and motility (Holst and Orskow, 1994). Somatostatin is synthetized from duodenal endocrine cells and from pancreatic D-cells (Brazeau et al., 1973). Chyhololecystokin-8 (CCK-8) is conveyed from the duodenum in response to the presence of digested food, particularly fatty acids and amino acids (Wilding, 2002). Serotonin has been proved to influence crypt epithelial secretion and proliferation (Gershon and Tack, 2007). Secretin is found in S cells which gradually decrease from duodenum to jejunum. The free hydrogen ions from duodenum are the strongest secretin stimulators. In addition, salts and alcohol stimulate acid secretion and increase secretin secretion (Dezfuli et al., 2000; Dezfuli et al., 2002). Substance P (SP) is a neuropeptide that performs some tasks such as neurotransmitter and neuromodulator (Wang et al., 1985). Histamine is a decarboxylative form of histidine and a potent vasodilator in animal tissues. Histamine affects 4 types of receptors (H1R, H2R, H3R, H4R) and transmits signal into the cell via G protein (Kalpaklıoğlu et al., 2012).

The purpose of this study was to examine regional and mucosal distributions of glucagon, somatostatin, CCK-8, serotonin, secretin, SP and histamine in the intestinal endocrine cells of New Zealand white rabbit (Orzyctolagus cuniculus) using immunohistochemistry.

Materials and Methods

Ten adult male New Zealand white rabbits were used in immunohistochemical study. The animals were provided from particular farms and were maintained in proper condition after requisition. The animals were euthanized with the approval of the Animal Experiments Ethics Board of Suleyman Demirel University (SDU-HUDAL B.30.2.SDU.0.05.06.00-186). The animals were anesthetized with intramuscular 35 mg/kg ketamine and 5 mg/kg xylasine (Sanford and Colby, 1980). After anaesthetizing, the abdominal cavities of the animals were opened. Samples harvested from the small (duodenum, jejunum and ileum) and large (cecum, distal colon, proximal colon and rectum) were fixed in Bouin’s solution for 15 h. After paraffin embedding, 5-6-µm-thick sections were taken at intervals of every 60 min from 5 pieces of the paraffin blocks. Sections were deparaffinized, rehydrated and stained immunohistochemically using the PAP method (Sternberger, 1986). According to this method, sections were washed...
Results

Regional and mucosal distributions of IR cells in the small and large intestine sections are given in Table 2.

The immunohistochemical results disclosed the regional and mucosal distribution of endocrine cells that secrete glucagon, somatostatin, CCK-8, serotonin, secretin, SP and histamine in the small intestine (duodenum, jejunum, and ileum) and large intestine (cecum, proximal colon, distal colon, and rectum) of New Zealand white rabbit. Cells in the intestines were occasionally spindle shaped with long cytoplasmic extension at the end of the lumen (open-type cells), while generally, cells of oval shape (closed-type cells) were also identified in the intestinal gland areas. Generally, open cell types with apical cytoplasmic processes that attained the glandular or intestinal lumen of the small and large intestinal epithelium were observed. Closed-type cells settle on the basis of the digestive tract epithelium and can not extend to the lumen of the organ. No immunoreactivity was shown in the negative control sections.

Immunoreactive cells for glucagon, somatostatin, serotonin, secretin and SP were found in LE of the small and large intestine.

Glucagon-IR cells were observed in the small and large intestine sections. In the small intestine, few glucagon-IR cells were found in the lamina epithelialis of duodenum, jejunum and ileum (Fig. 1a), but then none were detected in the Brunner’s gland. In the large intestine, these cells were moderate in number in the distal (Fig. 1b) and rectal gland regions and then decreased in the intestinal epithelia.

Somatostatin-IR cells were determined throughout small and large intestine. Also, few IR cells were found for somatostatin in the Brunner’s glands. The number of somatostatin-IR cells in the duodenum were found to be moderate, but few were observed in LE of jejunum and ileum. A few of these cells were identified in LE and gland of large intestine regions (Figs. 1c-d).

Numerous CCK-8-IR cells were present in the...
Fig. 1: Distribution of glucagon and somatostatin IR cell. (a) Ileum. The glucagon-IR cell in the lamina epithelialis (arrow), (PAP, bar: 50 µm). (b) Distal colon. The glucagon-IR cell in gland (arrow), (PAP, bar: 50 µm). (c) Proximal colon. The somatostatin-IR cell in the lamina epithelialis of the crypt (arrow), (PAP, bar: 50 µm). (d) Distal colon. The somatostatin-IR cells in glands (arrows), (PAP, bar: 50 µm)

Fig. 2: Distribution of the CCK-8-IR cell in the lamina epithelialis of the villus (arrow) (a) duodenum and the glands (arrow) of the (b) distal colon and (c) rectum (PAP, bar: 50 µm). (d) Distribution of the serotonin-IR cell in the lamina epithelialis of the cecum crypt (arrow) (PAP, bar: 50 µm)
duodenal epithelia (Fig. 2a) but few in the jejunum and ileum regions. These cells were detected numerously in the distal (Fig. 2b) and rectal glands (Fig. 2c), but were absent in the proximal colon and cecum regions. Serotonin-IR cells were detected very numerous throughout the duodenum, jejenum and ileum, although none were found in the Brunner’s gland. These cells were observed throughout the whole large intestine regions at various frequencies (Figs. 2d and 3a).

Secretin-IR cells were restricted to the gastrointestinal tract with rare and few frequencies. These cells were rarely found in the duodenum epithelium (Fig. 3b) but none were found in the Brunner’s gland region. These cells were rarely observed in the large intestine regions while the cells were not found in the cecum, proximal colon or rectum glands. It was noticed that cells in the lamina epithelialis of small and large intestine is linked to the lumen.

SP-IR cells were rarely identified in the basal portion of the duodenum (Fig. 3c) but none were defined in the Brunner’s gland portion. These cells were also spotted in the middle portion of the jejunum and the ileum. These cells were identified in the large intestine epithelium and glands, but those cells were not observed in the cecum glands.

Histamine-IR cells were only observed in the duodenal section of the small intestine where they were present at a rare frequency in the intestinal epithelia. The cells were not found in the Brunner’s glands, jejunum or ileum. No IR cells were observed in the cecum and proximal colon. However, a few number of histamine-IR cells were identified in the rectum (Fig. 3d). Histamine-IR cells in the lamina epithelialis of small and large intestine are linked to the lumen and the histamine-IR ones in rectal glands cannot reach the lumen.

Discussion

The present study identifies seven types of endocrine cells in the small and large intestine of the New Zealand white rabbit. These cells were IR for glucagon, somatostatin, CCK-8, serotonin, secretin, SP and histamine. In the intestinal regions, the spindle shaped-cells were generally opened-type cells, while generally oval shaped-cells were closed-type cells. The cell types and shapes detected in this study are similar to those reported in other mammals (Baltazar et al., 1998; Dall’Aglio et al., 1998; Ku et al., 2004; Santos et al., 2008; Lee et al., 2010; Adnyane et al., 2011).

Glukagon-IR cells were not found in the small and large intestine regions of SKH-1 hairless mice (Ku et al., 2002) and ddN mice (Lee et al., 2010), but these cells were determined numerous common tree shrew (Yamada et al., 1999). In this study, these cells were identified few in the small intestine regions and moderate number in the large intestine regions.
Our results showed that moderate number of somatostatin-IR cells were present in the epithelium of duodenum. But these IR cells were very numerous in the duodenum of the ostrich (Bezuidenhout and Van Aswegen, 1990) and one-humped camel (Althaiaia et al., 2012). In accordance with the results of this study, somatostatin-IR cells have been reported few in the large intestine regions of the musk shrew (Kitamura et al., 1990), common tree shrew (Yamada et al., 1999), ddN mice (Lee et al., 2010). Althaiaia et al. (2012) have been reported that detected very high level of expression at the Brunner’s glands thoughout the duodenum. In this study, cells IR for somatostatin were observed few in the Brunner’s glands.

Yaman et al. (2007) have reported that CCK-8-IR cells were not detected in small intestine regions of porcupine. CCK-8-IR cells were limited to the small intestine of cow, calf (Kitamura et al., 1985), lesser mouse deer (Agungpriyono et al., 1994). In the present study, these IR cells were numerous in the duodenal epithelia but few in the jejenum and ileum regions.

No CCK-8-IR cell was detected in the large intestine regions of wild boar (Dall’Aglio et al., 1998), common tree shrew (Yamada et al., 1999), SKH-1 hairless mice (Ku et al., 2002), barking deer (Adnyane et al., 2011) and New Zealand rabbit except for the distal colon and rectum of this study.

Serotonin-IR cells were detected numerous in duodenum New Zealand rabbit in a similar frequency to other vertebrates (Mimoda et al., 1998; Gençer Taraçtı et al., 2005; Karadağ Sarı et al., 2007; Yaman et al., 2007; Althaiaia et al., 2012).

Numerous serotonin-IR cells were identified distributed in the large intestine regions examined. These results are consistent with previous reports of studies in duck (Gülmez et al., 2003), chicks (Rawdon and Andrew, 1994), common tree shrew (Yamada et al., 1999) and one-humped camel (Althaiaia et al., 2012). In addition, these cells were identified moderate in the large intestine regions of the musk shrew (Kitamura et al., 1990), Philippine carabao (Baltazar et al., 1998), ddN mice (Lee et al., 2010). However, serotonin-IR cells were not found in the large intestine regions of the SKH-1 hairless mice (Ku et al., 2002).

Our results showed little distribution of secretin in the endocrine cells of the duodenum, similar to that of the musk shrew (Kitamura et al., 1990), ruminant (Mimoda et al., 1998), SKH-1 hairless mice (Ku et al., 2002) and grass lizard (Lee and Ku, 2004). However, the cells are distributed to moderate Philippine carabao (Baltazar et al., 1998), sheep (Mimoda et al., 1998) and babirusa (Agungpriyono et al., 2000). In addition, secretin-IR cells were found numerous in the duodenum of the domestic duck (Castaldo and Lucini, 1991), cow and barbary sheep (Mimoda et al., 1998) and common tree shrew (Yamada et al., 1999).

In this study, secretin-IR cells were found in the large intestine at low frequency. No immunoreactivity was demonstrated in the large intestine of the ostrich (Bezuidenhout and Van Aswegen, 1990), the King’s skink (Arena et al., 1990), Philippine carabao (Baltazar et al., 1998), the common tree shrew (Yamada et al., 1999), the babirusa (Agungpriyono et al., 2000), the red-eared slider (Ku et al., 2001) and the grass lizard (Lee and Ku, 2004).

SP-IR cells were found mainly in the small intestine of cow, calf (Kitamura et al., 1990), sheep (Mimoda et al., 2005) pig, cat, rat and the one-humped camel (Keast et al., 1984; Gronstad et al., 1985; Lundquist et al., 1990; Al Haj Ali et al., 2007). SP-IR cells have been identified in the large and small intestine of the ostrich (Bezuidenhout and Van Aswegen, 1990), these cells were present moderate in the small intestine. It has also been reported that SP-IR cells were found in the small and large intestine of the cow and calf (Kitamura et al., 1985), domestic duck (Castaldo and Lucini, 1991) and one-humped camel (Al Haj Ali et al., 2007). But no IR cells were obtained in these regions of the fresh water turtle (Gençer Taraçtı et al., 2005). In this study, SP-IR cells were rarely identified in the large and small intestine.

Histamine is a peptide that provides the vasodilation, stimulates the gastric acid secretion and modulates the gastrointestinal function. Another important site for histamine storage and release is the enterochromaffin-like cell of the stomach (Köse and Hall, 2000; Solcia et al., 2000).

This peptide also helps digestion by increasing gastric and intestinal smooth muscle contraction (Bhagavan, 1992). Histamine-IR cells were numerous in the LE and moderate in the intestinal glands of the small and large intestine regions of the guinea pig (Tahara et al., 2000). In this study, these cells were only detected in the duodenal section of the small intestine where they were found with rare frequency in the intestinal epithelia. No IR cell was observed in the cecum and proximal colon. However, a small number of histamine-IR cells were identified in the rectum.

In conclusion, the immunohistochemical study shows that gastrointestinal tract of this species contained different types of endocrine cells similar to those found in other vertebrate species. However, some species-dependent unique distributions and frequencies of endocrine cells were also observed in the present study.

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