The distribution of glucagon-like peptide-1 (GLP-1)-containing endocrine cells was investigated in the chicken intestine by using immunohistochemical and morphometrical techniques. GLP-1-immunoreactive cells were mainly distributed in the whole jejunum and ileum, and rarely found in ascending duodenum, but not in other intestinal regions. These cells had pyramidal or spindle-like shape and a cytoplasmic process reaching to the intestinal lumen. In jejunum immunoreactive cells were mainly observed in the middle part of villi, and in ileum they were mainly found in the lower part of villi and crypts. The frequencies of occurrence of GLP-1-immunoreactive cells in the proximal, middle and distal jejunum were $16.69 \pm 7.47$, $22.06 \pm 10.13$ and $35.88 \pm 15.17$, respectively (cell numbers per mucosal area: cells/mm$^2$, mean $\pm$ SD), and those in the proximal, middle and distal ileum were $41.37 \pm 15.05$, $53.84 \pm 17.57$ and $73.12 \pm 18.46$. There were significant differences between two adjacent regions in jejunum and ileum of small intestine. These data shows that GLP-1-immunoreactive cells are distributed more densely in the distal small intestine, and GLP-1 may play an important role in this intestinal region.

**Key words**: chicken, glucagon-like peptide-1, immunohistochemistry, intestine, morphometry

---

**Introduction**

Glucagon-like peptide-1 (GLP-1) is identified as an “incretin hormone” with potent insulin-releasing activity, and much attention has been paid to the potential therapeutic aspects of GLP-1. In addition to the insulin-releasing activity this hormone has important biological actions, such as reduction of food intake (Furuse *et al.*, 1997) and deceleration of gastric emptying (Nauck, 1998, Schirra *et al.*, 2005).

GLP-1 is a product of the proglucagon gene and secreted by L cells in the intestine (Orskov *et al.*, 1987; Damholt *et al.*, 1998; Nauck, 1998). In mammals, immunohistochemical studies have shown that immunoreactive cells against GLP-1 antiserum are distributed in the whole intestine (Kauth and Metz, 1987; Fridolf *et al.*, 1991; Eissele *et al.*, 1992; Damholt *et al.*, 1999). Most cells showing immunoreactivity for
enteroglucagon (EG), which seems to be co-stored with GLP-1 in the same secretory granules of L cells (Varndell et al., 1985), were located in the mammalian colon and rectum (Calingasan et al., 1984; Agungpriyono et al., 1994). On the other hand, EG-immunoreactive cells are differently distributed in the intestine of avian species. For example, they are located in the upper alimentary tract in chicken (Rawdon, 1984; Yamanaka et al., 1989) and ostrich (Bezuidenhout and Van Aswegen, 1990). Our previous study has shown that GLP-1-immunoreactive cells were observed only in the small intestine of two avian species, chicken and ostrich (Hiramatsu et al., 2003). This finding suggests the important role of GLP-1 in the chicken small intestine. More detailed study on the distribution of endocrine cells containing GLP-1 is, however, needed to understand the role of this hormone in the control of the intestinal motility.

In the present study, we aimed to clarify the distribution of GLP-1-immunoreactive cells in the chicken alimentary tract by using morphometrical method.

Materials and Methods

Male White Leghorn chickens (n=6, 7 weeks), weighing about 700 g on average, were used in this study. Chickens were kept in our laboratory under controlled light condition (12 hrs light : 12 hrs darkness) and given a commercial diet and water ad libitum. They were sacrificed by decapitation under anesthesia. Tissue samples about 2 cm long were rapidly dissected out from each bird. Samples were taken from descending and ascending duodenum, proximal, middle and distal jejunum and ileum, colorectum and proximal and middle regions of cecum. Chickens were treated in accordance with the “Guideline for Regulation of Animal Experimentation (1997)” of Faculty of Agriculture, Shinshu University.

Immunohistochemistry

Tissue samples washed with 0.75% NaCl solution were immersed in Bouin’s fluid overnight at room temperature. They were embedded in paraffin wax according to standard procedures. Sections were cut at 5 µm thickness and used for for the detection of GLP-1 by immunohistochemical technique. The streptavidin-biotin method (Guesdon et al., 1979) was applied to detect GLP1-immunoreactive cells according to the procedure previously described (Hiramatsu and Ohshima, 1995). Rabbit antiserum against synthetic GLP-1 [1–19] conjugated to bovine serum albumin (Affiniti Research Products, UK, No.GA1176, diluted to 1 : 2,500) was used as the primary antibody in this study. This antiserum did not cross-react with other proglucagon-derived peptides (Tachibana et al., 2005). After counterstaining with Mayer’s hematoxylin, sections were mounted and observed under light microscope. Control sections were incubated with the normal rabbit serum instead of the specific primary antiserum or in the absence of the primary antiserum. They showed negative immunoreactivity.

Morphometry

To evaluate the frequency of occurrence of GLP-1-immunoreactive cells in each intestinal region, we only counted the immunoreactive cells with clearly identifiable nuclei, and the area of the mucosal layer was measured. The cell number per area of the mucosal layer (cells/mm²) was then calculated. This quantification was carried out by
the use of a computerized image analyzing system (KS400, ZEISS, Germany). Twenty areas were measured in each intestinal region of each bird. One hundred and twenty areas in total were measured in each intestinal region from six chickens. Statistical analyses were performed to assess the difference in the frequency of occurrence of GLP-1-immunoreactive cells between two adjacent intestinal regions using Student’s t-test.

Results

Many endocrine cells showing immunoreactivity for GLP-1 antiserum were observed in the whole jejunum and ileum. They were rarely found in the ascending duodenum, and not in other intestinal regions. In the proximal and middle jejunum, GLP-1-immunoreactive cells were mainly observed in the middle part of intestinal villi, but fewer in crypts than other intestinal regions (Fig. 1). In the middle and distal ileum, many of them were observed in the lower part of villi and crypts (Fig. 2). GLP-1-immunoreactive cells were pyramidal or spindle-like shape in the villous epithelium (Figs. 3 and 4), and comma-like shape in crypts (Fig. 5). These cells had a cytoplasmic process reaching to the intestinal lumen (Fig. 4, arrow).

The frequencies of occurrence of GLP-1 immunoreactive cells in each intestinal region are summarized in Fig. 6. In jejunum, the frequencies of GLP-1-immunoreactive cells in the proximal, middle and distal regions were $16.69 \pm 7.47$, $22.06 \pm 10.13$ and $35.88 \pm 15.17$, respectively (cell numbers per mucosal area : cells/mm$^2$, mean $\pm$SD), and in ileum those were $41.37 \pm 15.05$, $53.84 \pm 17.57$ and $73.12 \pm 18.46$. There were significant differences between two adjacent regions of the intestine. The frequency of occurrence of GLP-1-immunoreactive cells was increased with going to the distal region of the small intestine. The frequency in ascending duodenum was less than 0.01 cells/mm$^2$.

Discussion

Many regulatory peptides from endocrine cells in the mucosal epithelium of the alimentary tract as well as from neurons in the enteric nervous system are involved in the control of the intestinal motility (Dockray and Walsh, 1994). GLP-1 secreted from L-cells is one of such peptides and stimulates insulin-release from pancreatic B cells (Nauck, 1998). Our previous study has demonstrated that GLP-1-immunoreactive cells are found in the mucosal epithelium of chicken small intestine and their frequency of occurrence in ileum is significantly higher than that in jejunum (Hiramatsu et al., 2003). The present study showed the detailed distribution of GLP-1-immunoreactive cells in the chicken intestine; a continuous increase of these cells was found from the proximal jejunum to the distal ileum showing the highest density. There is the species difference in the distributional pattern of EG- or GLP-1-containing cells even in the mammalian alimentary tracts. The highest density of these cells occurs in the distal ileum of rat (Eissele et al., 1992) and dog (Damholt et al., 1999). In the contrast, the highest density of cells showing glicentin (which is also derived from proglucagon in intestinal L-cells) or GLP-1 immunoreactivity is shown in the rectum of sheep (Calingasan et al., 1984), man and pig (Eissele et al., 1992). It is, however, common in the mammals that
Fig. 6. Frequencies of occurrence of GLP-1-immunoreactive cells in proximal (prox.), middle (mid.) and distal (dist.) jejunum and ileum of the chicken. There is a significant difference between two adjacent regions of the small intestine.

\( p < 0.05 \) : between a and b, c and d. \( p < 0.01 \) : between b and c, d and e, e and f.
endocrine cells showing immunoreactivity for peptides related to the proglucagon-gene are distributed in the whole intestine (Calingasan et al., 1984; Eissele et al., 1992; Damholt et al., 1999; Van Ginneken et al., 2002).

Saito et al. (1989) have indicated that EG-immunoreactive cells are distributed in the whole intestine except duodenum and most dominant in colorectum of the domestic pigeon. This avian species shows similar distributional pattern to that of mammalian species. Our previous (Hiramatsu et al., 2003) and present studies reveal that GLP-1-immunoreactive cells are found mainly in jejunum and ileum and show the highest density of occurrence in the distal ileum. L-cells are the open-typed endocrine cells and releases GLP-1 in response to the chemical signals such as glucose, amino acids or a change of pH in the intestinal lumen. Ileal GLP-1-immunoreactive cells are perfectly positioned to receive these chemical signals derived from the diet (Eissele et al., 1992). In fact, the frequency of occurrence of GLP-1-immunoreactive cells is reduced in ileum of the chickens fed with the diet containing the low protein level (Hiramatsu et al., in preparation). It is probable that GLP-1 is released in response to the nutrients in the intestinal lumen and controls the motility of the ileal segment of the chicken intestine.

In the present study, it became clear that there was a difference in the distributional pattern of GLP-1-immunoreactive cells between jejunum and ileum of the chicken. In other words, immunoreactive cells for GLP-1 were mainly observed in the middle part of intestinal villi of jejunum, but in the lower part of intestinal villi and crypts of ileum. It has been shown that EG- and GLP-1-immunoreactive cells are mainly found in the crypts and lower villi of the chicken (Yamanaka et al., 1989) and mouse (Aiken et al., 1994) small intestine respectively. Because the migration of entero-endocrine cells as well as other types of epithelial cells occurs along the crypt column (Tsubouchi, 1981), our data of this study may indicate the active proliferation of GLP-1-immunoreactive cells in the ileal segment of the chicken intestine. More systematic studies, however, are necessary to investigate this phenomenon.
In conclusion GLP-1-immunoreactive cells show the highest frequency of occurrence in the posterior part of ileum and GLP-1 may play an important role in this intestinal segment of the chicken.

References


Saito T, Yamada J, Kitamura N and Yamashita T. An immunohistochemical study on the


