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Immunohistochemical Distribution of Somatostatin, Glucagon and Gastrin in the Gastric Fundus of the Citellus (*Spermophilus xanthoprymnus*)

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Abstract: The distribution of endocrine cells in the fundus of the citellus (*Spermophilus xanthoprymnus*) was investigated using immunohistochemical PAP (Peroxidase-Antiperoxidase) method. Three types (somatostatin, glucagon, gastrin) of immunoreactive endocrine cells were identified in this study. They were spherical or spindle-shaped. Moderate number gastrin immunoreactive cells were identified in the fundus. Closed type gastrin immunoreactive cells were exclusively located in the basal portion of the fundic region, but occasionally open type cells were found mixed with closed type cells. Somatostatin immunoreactive cells were restricted to the fundus with few and moderately frequencies. They were dispersed in the whole gastric mucosa, between chief and parietal cells. However, closed type cells were exclusively located in the basal portion of the fundic mucosa, but occasionally open type cells were found mixed with close type cells. Closed type glucagon immunoreactive cells were restricted to the basal portion of the fundic mucosa with a few frequencies. Immunocytochemical localization of the glucagon, gastrin and somatostatin in the gastrointestinal tract of the *Spermophilus xanthoprymnus* was described for the first time in the present study. This study showed similarity to that observed in the rat and mammalian species.

Key words: Anatolian souslik, immunohistochemistry, fundus, gastrin, somatostatin, glucagon

INTRODUCTION

Gastrointestinal hormones are secreted by endocrine cells, which are distributed throughout the mucosa of the gastrointestinal tract. They have important functions in the overall regulation of digestive processes such as nutrient absorption, the secretion of intestinal and fundic glands (Deveney and Way, 1983). Endocrine cells play an important role in the gastrointestinal tract. Many different cell types have been identified, which produce a variety of biologically active peptides (Solcia *et al*., 1987). Until, now the investigation of gastrointestinal endocrine cells is considered to be an important part of a phylogenic study (D'Este *et al*., 1994). In addition, the regional distributions and relative frequencies of these endocrine cells were varied with animal species and feeding habits (Solcia *et al*., 1975). Different gut hormones have a significant role on the digestive functions of the stomach and intestine (Fujita and Kobayashi, 1977). The distribution of endocrine cells in the gastrointestinal tract has been widely investigated in the gastrointestinal tract of many domestic (Castaldo and Lucini, 1991; Ceccarelli *et al*., 1985; Mimoda *et al*., 1998; Nisa *et al*., 2005) and wild animals (Krause *et al*., 1985). The distribution and relative frequency of endocrine cells in the gastrointestinal tract was demonstrated in the gerbil (Lee *et al*., 2000), hairless Mouse (Ku *et al*., 2002), BALB/c Mouse (Ku *et al*., 2004) homozygous obese mouse (Spangeus and El-Salhy, 1998; Spangeus *et al*., 1999) and the Korean tree squirrel (Lee *et al*., 1991).

In addition, morphological studies at light and electron microscopic level have demonstrated the presence and distribution of hormone-producing endocrine cells in the gastrointestinal tract of man and domestic mammals (Facer *et al*., 1985; Rindi *et al*., 1986; Kawakita *et al*., 1990; Agungpriyono *et al*., 2000).

The Anatolian ground squirrels (*Spermophilus xanthoprymnus*), which are Asia Minor ground squirrels, are from the Sciuridae family, which was described in the range from Anatolia up to the Caucasus (Wilson and Reeder, 1993).

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The purpose of the present study was to clarify the distribution of somatostatin, glucagon and gastrin immunoreactive cells in the fundus of the Anatolian ground Squirrel using immunohistochemistry, to provide additional information on the distribution of its digestive system.

MATERIALS AND METHODS

Four adult Anatolian souslik (*Spermophilus xanthoprymnus*) trapped in Aksaray that is in the central Anatolian region were used in this study. The animals were anesthetized and killed using ether. Small pieces of tissues were dissected from the gastric fundus was removed immediately and placed in 10% formalin in Phosphate-Buffered Saline (PBS), pH 7, 4, for 18 h before paraffin embedding. Tissue samples were routinely processed through a graded series of alcohols, cleared in xylol and embedded in paraffin. Sections were cut 5 μm thickness, mounted on gelatin-coated glass slides and stained with immunohistochemically method.

Immunohistochemical staining was carried out by using the Peroxidase-Antiperoxidase (PAP) method (Bourne, 1985). Endogenous peroxidases were blocked of activity in 0.08% Hydrogen peroxide (H_2O_2) in methanol (10 min). After rinsing in 0.02 M Phosphate Buffered 0.1 M Saline (PBS) and in order to the block unspecific binding, an incubation with (1:10) normal goat serum in 0.1 M, PBS, pH 7.2 was performed.

Sections were incubated for 16-20 h at 4°C in rabbit anti-glucagon (Sigma), rabbit anti-somatostatin (Sigma), rabbit anti-gastrin (Sigma). Antibodies was diluted to 1:200, 1:500, 1:1000 with PBS containing 0.25% sodium azide and 2.5% bovine serum albumin, respectively. Sections were then incubated in goat anti-rabbit Ig G (Dako), followed by rabbit peroxidase anti-peroxidase complex (Sigma), both at dilution of 1:50 in PBS for 1 h at room temperature. Sections were washed in PBS for 30 min after each incubation and finally immersed in glucose oxidase-Diamino Benzidine (DAB), nickel ammonium sulphate (GDN) substrate (Shu *et al*., 1988) for 10 min. After washing in distilled water, sections were counter stained with eosin. Sections were dehydrated and coverslips mounted with mounting medium. Sections were examined with light microscope and photographs were taken.

RESULTS

Immunoreactive endocrine cells for somatostatin, glucagon and gastrin were identified in the fundus of the *Spermophilus xanthoprymnus* in this study. Most of the endocrine cells were located in the basal portions of the glands. The immunoreactive cells were round or spindle in shape. The immunoreactive cells were of either open or closed type. They appeared as close-type cells as they did not possess lamina contact with their apical cytoplasmic processes and distributed at the bases of the glands.

Somatostatin immunoreactive cells were restricted to the fundus with few and moderately frequencies. They were dispersed in the whole gastric mucosa, between chief and parietal cells (Fig. 1). However, closed type cells were exclusively located in the basal portion of the fundic mucosa, but occasionally open type cells were found mixed with close type cells. Closed type glucagon immunoreactive cells were restricted to the basal portion of the fundic mucosa with a few frequencies (Fig. 2). Moderate number gastrin immunoreactive cells were identified in the fundus. Closed type gastrin immunoreactive cells were exclusively located in the basal portion of the fundic region, but occasionally open type cells were found mixed with closed type cells (Fig. 3).

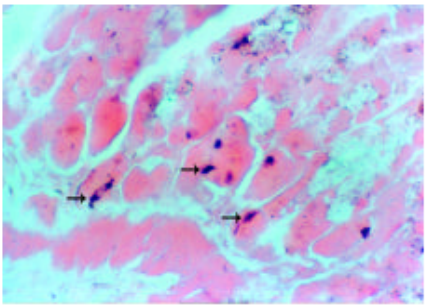

Fig. 1: Photomicrograph illustrating, the distribution of cells immunoreactive for somatostatin in the fundic gland region of the *Spermophilus xanthoprymnus* (arrows), 20×5

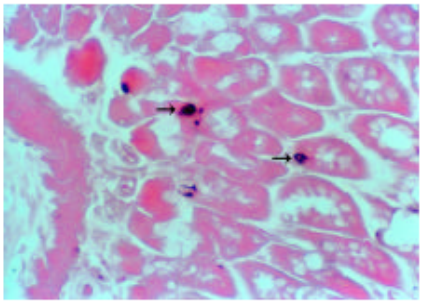

Fig. 2: Photomicrograph illustrating, the distribution of cells immunoreactive for glucagon in the fundic gland region of the *Spermophilus xanthoprymnus* (arrows), 20×5

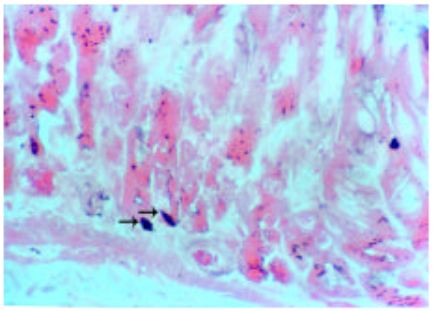

Fig. 3: Photomicrograph illustrating, the distribution of cells immunoreactive for gastrin the fundic gland region of the *Spermophilus xanthoprymnus* (arrows), 20×5

DISCUSSION

It is well known that regulation of the motility, secretion and absorption of the fundus is coordinated by neural and hormonal controls (Stevens and Hume, 1995). Gastrin is the first peptide to be purified sufficiently and has been localized to specific endocrine-type cells called G cells that lie midway between the neck and the base of the antrals glands (Solcia *et al*., 1987). Some hormones and neurotransmitters, such as Gastrin-Relasing Polypeptide (GRP), stimulate the release of gastrin (Walsh, 1987), while others such as somatostatin, inhibit release (Creutzfeldt and Arnold, 1978). In the present study, gastrin immunoreactive cells were detected in the fundus of *Spermophilus xanthoprymnus*. Although, no gastrin immunoreactivity in the gastric fundus that gastrin immunoreactive cells were only detected in the pyloric gland region and intestinal region of Nude Mice (Ku *et al*., 2006), Malayan Pangolin (Nisa *et al*., 2005), BALB/c Mouse (Ku *et al*., 2004), Tree Shrew (Yamada *et al*., 1999), *Babyrousa babyrussa* (Agungpriyono *et al*., 2000). However, in the gastrointestinal tract of Manchurian chipmunk, gastrin immunoreactive cells were detected from the fundus to ileum (Lee *et al*., 1997). These differences might be due to different antisera, methods and species used in each study (Walsh, 1987).

Somatostatin consisting of 14 amino acids was isolated from hypothalamus of sheep for the first time and it could be divided into a straight form and a cyclic form. This substance inhibits the secretion of the other neuroendocrine hormones (Brazeau *et al*., 1973). It is known that somatostatin immunoreactive cells show the widest distribution in the whole gasrtrointestinal tract except for the large intestine of all vertebrate species investigated. In the gastrointestinal tract of Manchurian chipmunk, they were detected somatostatin immunoreactive cells (Lee *et al*., 1998), but they were restricted to the pylorus of the gerbil (Lee *et al*., 2000). In mice strains, they were observed form the fundus to ileum of hairless Mouse (Ku *et al*., 2002), Nude Mice (Ku *et al*., 2006), Malayan Pangolin (Nisa *et al*., 2005), BALB/c Mouse (Ku *et al*., 2004), Tree Shrew (Yamada *et al*., 1999). In the present study, Somatostatin immunoreactive cells were detected throughout in the fundus.

Glucagon is synthesized in the A cells of the pancreas and regulates serum glucose levels. These immunoreactive cells have been demonstrated in various mammals. They were demonstrated in the whole gastrointestinal tract of the common tree shrew (Yamada *et al*., 1999). Baltazar *et al*. (1998) insisted that these immunoreactive cells only detected in the intestinal tract of the carabao and Lee *et al*. (1991) reported that they were restricted to the stomach regions of the Korean tree sguirrel. In addition, glucagon immunoreactive cells were detected in the fundus Balb/c-nu/nu (Ku *et al*., 2006); Balb/c Mouse (Ku *et al*., 2004); Babirusa (Agungpriyono *et al*., 2000). In another study, on the *Spermophilus xanthoprymnus* that stated by us serotonin immunoreactive cells were distributed throughout the fundus ventriculi and duodenum (Timurkaan *et al*., 2009).

Endocrine cells in the alimentary tracts appear remarkably different, depending on the regional distribution, relative frequency, cell types with animal species and each regional part of the gastrointestinal tract. In addition, many studies have elucidated the regional distribution and relative frequency of different endocrine cells in the gastrointestinal tract of various vertebrates including various species of rodents (Spangeus *et al*., 1999).

CONCLUSION

In order to obtain a better understanding of the functional role of glucagon, gastrin and somatostatin, we investigated their immunocytochemical localization in the gastrointestinal tract of *Spermophilus xanthoprymnus*. Immunocytochemical localization of the glucagon, gastrin and somatostatin in the gastrointestinal tract of the *Spermophilus xanthoprymnus* was described for the first time in the present study. This study showed similarity to that observed in the rat and mammalian species. The results of the present study may contribute to extension of data in this field of science.

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