Acta histochemica 111 (2009) 83–92

Available online at www.sciencedirect.com
ScienceDirect

acta histochemica
www.elsevier.de/acthis

SHORT COMMUNICATION

An immunocytochemical study of the endocrine cells in the stomach and duodenum of *Zonotrichia capensis subtorquata* (Passeriformes, Emberizidae)

Rosa Maria Marcos Mendes*, Aparecida Alves do Nascimento, Clarice Machado Dos Santos, Tânia Regina Dantas Cardoso, Nadja Lima Pinheiro, Armando Sales

Laboratório de Histologia e Embriologia, Animal Biology Department, Instituto de Veterinária, sala 85. Universidade Federal Rural do Rio de Janeiro, Rod. BR 465, Km 07, CEP 23.890-000, Seropédica, Brazil

Received 10 December 2007; received in revised form 21 February 2008; accepted 28 February 2008

KEYWORDS
Immunoreactive cells;
Endocrine cells;
Stomach;
Duodenum;
Bird

Summary

The main purpose of this study was to evaluate the regional distribution pattern and relative frequency of some endocrine cells in the three portions of the gastrointestinal tract (GIT) – the proventriculus, gizzard and duodenum- of the rufous-collared sparrow (*Zonotrichia capensis subtorquata*), by immunohistochemical methods using six types of polyclonal antisera, specific for serotonin (5-HT), somatostatin (D cells), glucagon, motilin, polypeptide YY (PYY) and insulin. In the proventriculus, endocrine cells immunoreactive for all of these markers were observed. The somatostatin-immunoreactive cells were found with greater frequency, with the presence of cytoplasmic processes. In the gizzard, endocrine cells secreting somatostatin, 5-HT and PYY were detected, while those secreting glucagon and insulin were not. In the final part of the gizzard, endocrine cells secreting 5-HT were more frequent, and cells secreting somatostatin and insulin were not detected. All of the cell types studied were observed in the duodenum in different frequencies, except for cells immunoreactive for glucagon and insulin. The somatostatin-positive (D cells) were the most numerous, being more prevalent in the intestinal glands. The other endocrine cells were identified in smaller numbers, some of them located in the intestinal villi and Lieberkuhn glands. The finding of these cell types in the duodenum confirms their preferential location in the final portions of the principal segments of the digestive system and suggests control by

*Corresponding author. Tel.: +21 2682 1210x210.
E-mail address: r.mendes@superig.com.br (R.M.M. Mendes).

0065-1281/$ - see front matter
doi:10.1016/j.acthis.2008.02.003

feedback of its functions. In conclusion, some interesting distributional patterns of gastrointestinal endocrine cells were found in this species of sparrow.

Introduction

The gastrointestinal tract (GIT) is the body's largest endocrine organ and is the location of most of the cells of the diffuse neuroendocrine system already identified. These cells are distributed throughout the gastric, intestinal and pancreatic tissue (Swenson, 1988). Among the functions attributed to these endocrine cells are: control of the metabolism of carbohydrates and all the processes associated with digestion and absorption of nutrients, such as secretion of the glands; peristalsis; supply of blood; and reabsorption and kinetics of the epithelium of the GIT (Bloom et al., 1982; Creutzfeldt, 1984; Nicholl et al., 1985).

The digestion processes in the avian GIT depend on sophisticated control systems that co-ordinate secretion of digestive juices and movement of the luminal contents (Gulmez et al., 2003). Many studies have elucidated the regional distribution of different endocrine cells in the GIT of vertebrates, such as fishes (Ku et al., 2004), amphibians (Ku et al., 2003) and particularly mammals (Santos et al., 2008, in press). In birds, immunohistochemical studies of the stomach and intestine have indicated the presence of many types of endocrine cells, which (as in mammals) are immunopositive for serotonin and a range of regulatory peptides. Among the bird species studied are hatchling (Andrew, 1976; Monesi, 1960) and adult chickens (Martinez et al., 1991); quails (Polak et al., 1974); ducks (Castaldo and Lucini, 1991); Houbara bustards (Mensah-Brown and Lawrence, 2001); New Holland honeyeaters (Richardson et al., 1988) and pigeons (*Columba livia* var *domestica*) (Saito et al., 1989). These studies have concluded that the distribution of these endocrine cells varies among avian species according to their feeding habits (Yamada et al., 1979).

Recently, a variety of endocrine cells have been described in the pancreas of *Zonotrichia capensis subtorquata* (Nascimento et al., 2007), but there are no reports on the distribution of these cells in the GIT itself. The main purpose of this study was to evaluate the regional distribution pattern and relative frequency of the endocrine cells, by immunohistochemical methods, using antisera against serotonin, somatostatin, glucagon, motilin, polypeptide YY (PYY) and insulin in the proventriculus, gizzard and duodenum, to help clarify the functions of these cells.

Materials and methods

Collection of samples

Three adult rufous-collared sparrows (*Z. capensis*) were studied. The animals were donated to UFRRJ by the Brazilian Environmental Institute (IBAMA)/Flavio Xavier National Forest (FLONA)/Center for Reintroduction of Animals into the Wild (CETAS) in the municipality of Seropédica, Rio de Janeiro State, Brazil, through a technical-scientific cooperation agreement, because they were considered unable to survive in the wild. The birds were sacrificed with sodium thiopentone at a dose of 80 mg/kg. The experimental protocols described in the present study were performed in line with accepted ethical guidelines.

Tissue processing

Samples of the proventriculus, gizzard and duodenum were removed from each bird, cut into smaller fragments and fixed in Bouin's fluid (Di Fiori, 1975) for 24 h. After fixation, the tissues were dehydrated in a graded series of ethanol concentrations and processed for embedding in Paraplast (McCormick, St Louis, USA). About 5-μm thick sections were cut and mounted on glass slides precoated with 0.1% poly-L-lysine (Sigma Chemical Co., São Paulo, Brazil).

Immunohistochemistry

Sections were deparaffinized and rehydrated by routine protocols. They were incubated with methanol containing 0.3% H_2O_2 for 15 min to block any endogenous peroxidase. The sections were then incubated with a 1% solution of bovine serum albumin (B4287; Sigma) in phosphate buffered saline (0.01M, pH 7.4) for 30 min. Subsequently, they were labeled immunohistochemically using a three-layered avidin–biotin–peroxidase complex (ABC) method (Hsu et al., 1981) to identify specific endocrine cells. The sections were first incubated