Tissue and Cell 45 (2013) 338–349

Contents lists available at SciVerse ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Morphologic characterization and distribution of endocrine cells in the large intestine of the opossum *Didelphis aurita* (Wied-Neuwied, 1826)

Daiane Cristina Marques dos Santos[a,*], Marli do Carmo Cupertino[a], Rômulo Dias Novaes[a], Ítalo Augusto da Costa Soares[b], Cláudio César Fonseca[b], Sérgio Luis Pinto da Matta[a], Sirlene Souza Rodrigues Sartori[c]

[a] Department of General Biology, Federal University of Viçosa (UFV), Viçosa, MG, Brazil
[b] Department of Veterinary Medicine, Federal University of Viçosa (UFV), Viçosa, MG, Brazil
[c] Department of Animal Biology, Federal University of Viçosa (UFV), Viçosa, MG, Brazil

ARTICLE INFO

Article history:
Received 12 January 2013
Received in revised form 11 May 2013
Accepted 27 May 2013
Available online 28 June 2013

Keywords:
Insulin-immunoreactive endocrine cells
Argyrophil and argentaffin endocrine cells
Opossum
Histochemistry
Immunohistochemistry
Scanning electron microscopy

ABSTRACT

This study was designed to investigate the morphology and distribution of argyrophil, argentaffins, and insulin-immunoreactive endocrine cells in the large intestine of the opossum *Didelphis aurita*. Fragments of the large intestine of 10 male specimens of the opossum *D. aurita* were collected, processed, and submitted for histochemistry, immunohistochemistry, and scanning electron microscopy. The tunics of the large intestine of *D. aurita* presented morphological characteristics that have already been described for eutherian mammals. The morphometric data showed that the inner circular layer of all portions and regions analyzed is thicker relative to the longitudinal layer, and these layers in the rectum are thicker compared to the cecum and ascending colon. The majority of mucus-secreting cells have acid and neutral mucins, suggesting that the production of mucus is mixed. The number of these cells increases in the region of the cecum toward the rectum. Important findings include the occurrence of argyrophil, argentaffins, and insulin-immunoreactive endocrine cells in all segments of the large intestine of the opossum (*D. aurita*). To the best of our knowledge, this is the first report about the presence of insulin-immunoreactive endocrine cells in the large intestine of the opossum (*D. aurita*).

1. Introduction

The gastrointestinal tract plays a crucial role in the control of intake and absorption of nutrients, and its endocrine cells have an important regulatory mechanism, synthesizing hormones, activating and inhibiting neural circuits (Drucker, 2007). These cells constitute approximately 1% of the total intestinal epithelium and represent the largest population of hormone-producing cells in the body (Rehfeld, 1998). These cells show immunoreactivity for somatostatin, serotonin, cholecystokinin, gastrin, secretin, glucagon, motilin, histamine, gastric inhibitory peptide (Krause et al., 1989; Yaman et al., 2012), and insulin (Freitas-Ribeiro et al., 2011, 2012; Basile et al., 2012), among others.

The endocrine cells can be classified according to their morphology (Fujita and Kobayashi, 1977; Sjölund et al., 1983; Dayal et al., 1987), the ability to absorb certain salts (Grimelius and Wilander, 1980), the morphology of their secretory granules (Polak et al., 1993), and the presence of specific marker molecules (Rindi et al., 2004; Schönhoff et al., 2004).

In regard to the ability to absorb and reduce silver salts, endocrine cells are classified as argyrophil and argentaffins. In an argentaffin reaction (Barbosa et al., 1984), reducing the ammoniacal silver nitrate occurs through the reductive capacity of the intracellular components themselves. In an argyrophil reaction (Grimelius and Wilander, 1980), silver salts in an ammoniacal solution bind to the cytoplasmic granules and are reduced to metallic silver by exposure to an exogenous reducing substance.

Enteroendocrine cells can be distinguished by "open cells" with microvilli extending to the lumen and "closed cells" that do not reach the lumen. Their secretory products are accumulated in secretory granules and are secreted upon stimulation by exocytosis at the basolateral membrane into the interstitial space, where they can act locally or on distant targets through the bloodstream (Rehfeld, 1998; Dockray, 2003).

Insulin-producing cells have been identified in extrapancreatic regions such as the prostate (Stahler et al., 1988), nephron (Coutinho et al., 1985), central nervous system (Devaskar et al., 2002), retina (Meimaridis et al., 2003), and intestine (Coutinho et al., 1984; Bendayan and Park, 1991; Kendzierski et al., 2000;

* Corresponding author at: Universidade Federal de Viçosa, Departamento de Biologia Geral., Avenida Peter Henry Rolfs, s/n. Campus Universitário, Viçosa, Minas Gerais 36570-000, Brazil. Tel.: +55 31 3899 2515/3361; fax: +55 31 3899 2549.
E-mail addresses: daiane.santos@ufv.br, biomarques@vicosa.ufv.br (D.C.M. Santos).

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http://dx.doi.org/10.1016/j.tice.2013.05.003

Freitas-Ribeiro et al., 2011, 2012; Basile et al., 2012); however, there are no studies in the literature related to insulin-immunoreactive endocrine cells in the large intestine of marsupials, highlighting the necessity and importance of this research.

According to Rodrigues et al. (2005), morphological studies of the digestive tract are important as additional tools for studying physiological and pathological. Particularly, it is important to perform studies involving endocrine cells since the control of the motor and secretory events involved in digestion and absorption depend on the action of the endocrine system.

Moreover, these cells have an essential role in the control of food intake and in the repair and immunity of intestinal mucosae. They are also related to severe gastrointestinal disorders, especially irritable bowel syndrome postinfection, enteric infections, inflammatory bowel diseases (Moran et al., 2008), and neoplasia (Polak et al., 1993).

Therefore, further studies are needed regarding the endocrine cells to explore the therapeutic potential in diseases of the large intestine. Thus, how the digestive system works with didelphids is important because they promotes a histomorphometric database and provides better understanding of the digestive processes of wild mammals.

The marsupials of the genus *Didelphis* show special features that put them in evidence for research, among which the following are highlighted: their ancestral origin, considered as morphologically primitive mammals; their representing a link in the transition between Prototheria and Eutheria; their short gestation period since most of the fetal and embryonic development occurs outside the uterus, facilitating access to the various organs; besides varied eating habits. However, immunohistochemical studies of endocrine cells of marsupials are more focused on the pancreas, stomach, and small intestine (Krause et al., 1985; Barbosa et al., 1987; Krause et al., 1989; Takagi et al., 1990; Fonseca et al., 2002; Barbosa et al., 2006; Freitas-Ribeiro et al., 2011, 2012; Basile et al., 2012), with no work on the presence of these cells in the large intestine of *Didelphis aurita*.

In this context, this study was designed to investigate the morphology and distribution of argyrophil, argentaffins, and insulin-immunoreactive endocrine cells in the large intestine of the opossum *D. aurita*.

2. Materials and methods

2.1. Animals and ethical aspects

Ten male specimens of the opossum *D. aurita* weighing 1.03 ± 0.19 kg, crown-rump and snout-rump corporal lengths of 32.70 ± 3.59 and 46.05 ± 12.50 cm, perimeter and thoracic height of 22.70 ± 1.57 and 11.35 ± 0.78 cm, respectively, were used in this study. The animals were considered adults by having complete dentition (Macedo et al., 2006). Catches were authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, license 23204-1) in a small Atlantic forest area in the state of Minas Gerais, Brazil (S 20°45′14″, W 42°52′55″). The study was approved by the Institutional Ethics Committee for Animal Research (approval protocol 11/2010). Hooklike traps with dimensions of 75 cm × 31 cm × 31 cm were used with bananas and eggs as bait. After capture, the animals were kept in captivity for a period of 24 h, receiving water and food used in the capture, a diet similar to their natural feed (Carvalho et al., 2005). The animals were anesthetized with sodium thiopental, 30 mg/mL, using an intraperitoneal dose of 60 mg/kg. Euthanasia of the animals was performed under anesthesia through intracardiac administration of 0.25% potassium chloride, depending on the body weight.

2.2. Biometry and histological processing

After exposure of the abdominal cavity, the intestine was removed and placed in a flume, moistened with saline, allowing their measurement by caliper with no distention of the organ. The intestinal regions, cecum, colon (ascending, transverse, and descending), and rectum were identified by flexures and intestinally delimited by lashing. Three fragments were collected (proximal, middle, and distal) in each region, for a total of 15 fragments for each animal. The sections were fixed in 10% buffered formalin for 24 h, dehydrated in ethanol, cleared in xylene, embedded in paraffin, and sectioned at 5 μm thick at intervals of 50 μm in a rotary microtome (Leica Multicut 2045; Reichert-Jung Products, Germany).

2.3. Histochemistry, immunohistochemistry and histomorphometry

The histological sections used in the histological description and morphometric analysis were stained with hematoxylin and eosin (Bancroft and Stevens, 1996). Alcian blue, pH 2.5 (AB) (Bancroft and Stevens, 1996), and periodic acid-Schiff (PAS) methods (McManus, 1948) were used to detect acidic and neutral mucins, respectively. Acridine orange stains were used to detect mast cells (Kawasaki et al., 1991). Grimelius (Grimelius and Wilander, 1980) and modified Masson–Fontana (Barbosa et al., 1984) methods were applied for marking argyrophil and argentaffin enteroendocrine cells, respectively. Indirect immunoperoxidase (Sternberger, 1979) was used to detect insulin-immunoreactive endocrine cells, using positive and negative control sections of the pancreas from the opossum processed under the same conditions. The monoclonal antibodies used in the immunohistochemistry technique were purchased from Bethyl Laboratories (lot no. A90-117p-4). Photographic documentation of the preparations was performed under a CX31 light microscope (Olympus, Tokyo, Japan) with a SC020 digital camera (Olympus, Tokyo, Japan).

The morphometrical analysis of the intestinal tunics was made using the image analysis software Image-Pro Plus 3.5 (Media Cybernetics, Inc., Rockville, Maryland, USA). Ten random measurements were made from each one of the three portions (proximal, middle, and distal) of each intestinal segment (cecum; ascending, transverse, and descending colons; and rectum). We measured the height and thickness of 30 absorptive and mucus-secreting cells from each intestinal segment. Cells secreting acidic or neutral mucins were quantified in 10 random fields of 78,500 μm^2 using the same image analysis software. The results were converted to cells per mm^2.

2.4. Scanning electron microscopy

For scanning electron microscopy, fragments of the middle portion of the cecum, colon (ascending, transverse, and descending), and rectum of five animals were fixed in buffered formalin at 10% for 24 h. The fragments were dehydrated in ethanol, submitted to critical point drying (CPD030; Bal-Tec, Witten, North Rhine-Westphalia, Germany), mounted on stubs, metallized with gold, and examined in a scanning electron microscope (Leo 1430VP; Carl Zeiss, Jena, Thuringia, Germany).

2.5. Statistical analysis

The results were represented as mean and standard deviation (mean ± SD). The normality of data distribution was verified using the Shapiro–Wilk test. Considering the result of this test, the Kruskal–Wallis or Dunn's test was used for multiple comparisons. Results were considered significant when $p < 0.05$. All the tests were