Tissue and Cell xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Tissue and Cell

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

Influence of feeding habits in the endocrine pancreas of insectivore bat *Pteronotus personatus* and nectarivore bat *Anoura geoffroyi*: A comparative stereological and immunohistochemical study

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ARTICLE INFO

Article history:
Received 27 August 2016
Received in revised form 10 January 2017
Accepted 10 January 2017
Available online xxx

Keywords:
Pancreatic islets
Chiroptera
Endocrine cells
Histology

ABSTRACT

Pteronotus personatus as an insectivore bat and has a diet that consists of a high protein diet, whereas the diet of *Anoura geoffroyi*, a predominantly nectarivore bat, is rich in simple sugars like sucrose, glucose and fructose. Considering that diet influences the activation of different pathways, which may influence morphological adaptations in the gastrointestinal system, the aim of this study was to compare the morphology of the endocrine pancreas in *P. personatus* and A. *geoffroyi*. For this, histological, stereological and immunohistochemical methods were used. In *P. personatus*, the average diameter of the pancreatic islet was 40.47 μm ± 13.94, while in *A. geoffroyi* was 88.16 μm ± 36.40. The total number of pancreatic islets in *P. personatus* was 26150 ± 2346 and in *A. geoffroyi* was 15970 ± 1666. In *P. personatus*, the volume density of the pancreatic islets was 3.4%± 2.6, whereas in *A. geoffroyi* the volume density was 6.1% ± 3.7. In addition, the immunodensity of the α, β and δ cells, in *P. personatus* was 25.8% ± 11.9, 35.5% ± 13.5, 3.9% ± 0.7, respectively, and in *A. geoffroyi* was 33.10% ± 12.7, 55.08% ± 7.4, 6.2% ± 4.6, respectively. In conclusion, the results of this study indicate differences in the pancreatic weight/body, weight ratio, diameter and volume density of pancreatic islets and in immunodensity of the β and α cells between both species, which have different dietary habits.

1. Introduction

The order Chiroptera is composed of approximately 1120 species, including the placental mammals bats (Simmons, 2005). Mammals in the order Chiroptera have highly diversified eating habits, and this order includes insectivorous, nectarivorus, frugivorus, piscivorus and hematophagous bats. The large dietary variety among these species provides a unique opportunity to investigate the influence of diet on physiological and morphological features (Schondube et al., 2001). *Pteronotus personatus* (Wagner, 1843), known as Wagner's mustached bat, belongs to the family Mormoopidae, and is an insectivore that requires a high protein diet. *Anoura geoffroyi* (Gray, 1838), known as Geoffroy's tailless bat, belongs to the family Phyllostomidae and is predominantly a nectarivore. Nevertheless, *A. geoffroyi* is also able to consume fruit and pollen, and its diet is rich in simple sugars such as sucrose, glucose and fructose (Reis et al., 2007).

Diabetes is the most common disease of the endocrine system; therefore, the pancreas, which is important in regulating blood sugar levels, had been extensively studied in mammals. Moreover, chronic hyperglycemia is associated with dysfunction or loss of pancreatic β cells (Chen et al., 2011). Bats are important experimental animals, because they are more similar to humans in various morphological and biochemical aspects than are rats, which are commonly used in scientific experimentation, for having a gall bladder, encapsulated pancreas and hepatic distribution of the PEPCK enzyme (associated with neoglucogenesis) similar to those of humans (Pinheiro et al., 2006).

In Chiroptera, the endocrine pancreas has been analyzed in different species such as the insectivorous bats *Myotis lucifugus*

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http://dx.doi.org/10.1016/j.tice.2017.01.001
0040-8166/

(Bauman, 1990; Nunez et al., 1980), the fruit–eating bats *Artibeus aegyptiacus* and *Artibeus lituratus* (Pinheiro et al., 2006; Protzek et al., 2010), *Rousettus aegyptiacus* (Michelmore et al., 1998) and the hematophagous bats *Diphylla ecaudata* (Machado-Santos et al., 2013) and *Desmodus rotundus* (Freitas et al., 2013), using different morphological methods. These studies suggest that there is a close relationship between the distribution, arrangement and proportion of endocrine cells of the pancreatic islets, and the type of diet and/or phylogeny in bats.

For phyllostomid and mormoopid bats, the variability in morphology of the gastrointestinal tract may be related to shifts in dietary composition (Strobel et al., 2015; Pérez-Barbería et al., 2001; Machado-Santos et al., 2009; Santos et al., 2008; Langer, 2003; Schondube et al., 2001). Wetterer et al. (2000), mapping diets onto phylogeny, suggested that insect–eating bats represent the ancestral phyllostomid diet and that most diets evolved from insectivory. The insectivorous diets are correlated with a reduced ability to assimilate carbohydrates (other than trehalose), and a relatively high ability to assimilate protein (Hernandez and Martinez Del Rio, 1992). A change in diet from insects to nectar or fruit was accompanied by increased sugar intake and a reduction in protein and trehalose ingestion (Schondube et al., 2001).

Considering that diet influences the activation of different pathways, which may induce morphological adaptations in the gastrointestinal system, the aim of this study was to compare the morphology of the endocrine pancreas in *P. personatus* and *A. geoffroyi*.

2. Material and methods

The experimental procedures and animal care were approved by the institutional committee for animal use of Rio de Janeiro Federal Rural University, Seropédica, Brazil.

2.1. Capture of the bats and collecting material

Five adult males of each species were used, and collected according to Brazilian law. The specimens were collected during the night with mist nets and hand nets, in Casa de Pedra cave in the state of Sergipe, Brazil. Bats were transported to Federal University of Sergipe, where were weighed and euthanatized using sodium thiopental at a dose of 100 mg/Kg i.p. After that, median celiotomy was performed to remove the pancreas. The volume of the pancreas (V[p]) was measured by using Scherleís method (Scherle, 1970). After weighing, each pancreas was fixed in freshly prepared Bouin's liquid for 6 h and preserved in a solution of ethanol 70% and sent to Rio de Janeiro Federal Rural University to be processed according the standard histological technique for paraffin embedding. Five-micrometer thick serial slices were stained with hematoxylin-eosin (HE) (Lillie and Fullmer, 1976).

2.2. Immunofluorescence and immunohistochemistry

For immunofluorescence, antigen retrieval was accomplished using citrate buffer, pH 6.0, 60 °C for 20 min and blocked with ammonium chloride, 2% glycin, and phosphate buffer, pH 7.4 (PBS). Pancreatic sections were simultaneously incubated with rabbit anti-glucagon (A0565, Dako), guinea pig anti-insulin (A0564, Dako) and rabbit anti-human somatostatin (A0566, Dako). Primary antibodies were diluted to 1:50, 1:50 and 1:300 respectively in blocking buffer (PBS/1% BSA) and incubated overnight at 4 °C. Then the samples were incubated for 1 h at room temperature with fluorochrome-conjugated secondary antibodies: donkey anti-rabbit IgG-Alexa 488 and goat anti-guinea pig IgG-Alexa 546 (Invitrogen, Molecular Probes, Carlsbad, CA, USA), both diluted to 1:50 in PBS/1% BSA. After rinsing in PBS, the slides were mounted with DAPI nucleic acid stain and SlowFade Antifade (Invitrogen, Molecular Probes, Carlsbad, CA, USA). Double indirect immunofluorescence images were captured using a Zeiss model LSM 510 confocal laser scanning microscope (Meta, Germany). For immunohistochemistry, sections were incubated with anti-insulin (G 0785, Sigma-Aldrich) diluted to 1:1000, anti-glucagon (G 2654, Sigma-Aldrich) diluted to 1:2000 and anti-somatostatin (A0566, Dako) diluted to 1:300 and then amplified with a biotin-streptavidin complex (PK 6200; Vector). Insulin, glucagon and somatostatin were identified with 3,3'diaminobenzidine tetrachloride (H-2200, DAB, Vector) and sections were counterstained with Mayer hematoxylin. Digital images of the stained slices were obtained using a LC Evolution camera mounted on an Olympus BX51 microscope.

2.3. Pancreas morphometry

Five-micrometer thick sections were obtained from each pancreas and stained with hematoxylin-eosin (HE). From the digital images of pancreatic tissue, the smallest and largest diameters of each pancreatic islet were measured to calculate the average diameter of the islet (Image-Pro Plus version 7.0, Media Cybernetics, Silver Spring, MD, USA). At least, 100 islets were measured per animal.

2.4. Pancreas stereology

2.4.1. Islet number (N[islet])

The pancreatic islet number was estimated using a physical dissector-fractionator method (Bock et al., 2005). Briefly, in a consecutive series of sections, starting with a random section and leaving an interval of 10 sections, the distance between look-up and look-down sections was 20 μm for each pair, representing about 1/3 of the islet diameter in these animals. Thus, pancreatic islets seen in look-up in anterior sections but not the look-down sections were counted (Q_A^-), and the numerical density of islets (N_V) was estimated as: $N_V[islet] = Q_A^-/A_T{*}d$ ($1/mm^3$). The number of pancreatic islets (N[islet]) was estimated as the product: V[p]*Nv[islet] (Bock et al., 2005).

2.4.2. Islet volume density (Vv[islet]) and mass of islet (M[islet])

Vv[islet] was estimated by point-counting, the ratio of the number of points that hit the pancreatic islet (Pp) and the total number of test-points in a test-system made up of 36 test-points (P_T): $Vv[islet] = Pp[islet]/P_T$ (%). Subsequently, the volume was obtained by multiplying the Vv[islet] by pancreatic mass (Mandarim-de-Lacerda, 2003).

2.4.3. Volume density of the α, β, δ cells (Vv[α, β, δ cell]) and α, β, δ cell mass (M[α, β, δ cell])

Vv[α, β, δ cell] was estimated by image analysis using the density threshold selection tool applied to islets with insulin-positive areas. The volume density of the α, β, δ cells was expressed as a percentage of the pancreatic islet (Image-Pro Plus version 7.0, Media Cybernetics, Silver Spring, MD, USA). Thus, M [α, β, δ cells] was estimated as the product of Vv[α, β, δ cells] and M[islet] (Mandarim-de-Lacerda et al., 2010).

2.5. Statistical analysis

All the parameters analyzed were expressed as mean ± standard deviation (SD) and compared by unpaired *t*-test. The p-value <0.05 was considered statistically significant. The analyses were made in the software GraphPad Prism 5.

Please cite this article in press as: Machado-Santos, C., et al., Influence of feeding habits in the endocrine pancreas of insectivore bat Pteronotus personatus and nectarivore bat Anoura geoffroyi: A comparative stereological and immunohistochemical study. Tissue Cell (2017), http://dx.doi.org/10.1016/j.tice.2017.01.001