Autonomic Neuroscience: Basic and Clinical 177 (2013) 224–230

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

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

Ganglionar nervous cells and telocytes in the pancreas of *Octodon degus* Extra and intrapancreatic ganglionar cells and telocytes in the degus

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ARTICLE INFO

Article history:
Received 27 December 2012
Received in revised form 2 May 2013
Accepted 4 May 2013

Keywords:
Octodon degus
Caviomorph rodent
Pancreas
Ganglionar nervous cells
Enteric nervous system
Telocytes

ABSTRACT

This study shows for the first time the presence of intra and extrapancreatic ganglionar neurons and telocytes in *Octodon degus* such as those described in human and guinea pig pancreas. Pancreatic ganglionar neurons were identified by their histological characteristics as well as their positive immunostaining with mouse anti-human neuron specific enolase (NSE) antibody. Somatostatin secreting delta cells (D cells) in the islets of Langerhans were identified by positive immunostaining with rabbit antihuman polyclonal somatostatin antibody. Electron microscopy evidenced the presence of some unmyelinated axons in the interlobular spaces or septa, usually located adjacent to blood vessels and the exocrine epithelial ducts. The presence of telocytes with at least 2 telopodes was observed in the interlobular space, frequently in close spatial relationship with blood vessels and nerve endings. Telocytes were often observed in the vicinity or even in close proximity with both secretory acini and exocrine epithelial ducts and regulatory nerves and blood vessel apparatuses. A possible framework has been put forward within which such structures might contribute to elicit physiological responses in the pancreas. Further studies of synaptic interactions within and between pancreatic neuron cells are needed to help clarify the morphological results reported here. A broad overview of the field of neurogastroenterology with focus on the pancreas of *O. degus* related to the enteric nervous system (ENS) is provided in order to help design future studies on the connections of specific neurons forming pancreatic pathways, their neurotransmission processes and how disruption of these pathways may contribute to pancreatic disease.

1. Introduction

Octodon degus (degus) is a small diurnal caviomorph rodent native to Chile. The gestation period lasts 90 days and the average litter size is five pups. This animal adapts easily to animal facility conditions and has been used as an experimental model in a variety of studies regarding subjects such as placenta (Bosco, 1997; Bosco et al., 2007; Kertschanska et al., 1997; King, 1992), toxicology (Bosco, 2005; Bosco et al., 1997), diabetic eye cataract development (Nishi and Steiner, 1990), circadian rhythms (Lee, 2004), Alzheimer's disease (Inestrosa et al., 2005) and visual organization (Jacobs et al., 2003).

The degus has ordinary and high circulating glucose levels (Opazo et al., 2004), and its endocrine pancreas has unique alpha-cell crystals, a herpes-like virus, and islet amyloidosis (Spear et al, 1984). The molecular biochemistry of pancreatic hormones in degus (Hellman et al., 1990) and guinea pig (Iturriza et al., 1995) related to beta and alpha cells has been widely analyzed. Clear analogies, such as failure to stain alpha-cells using antisera against the C-terminal portions of the glucagon molecule, have been described for these two caviomorph species. Additionally, some studies have also demonstrated a number of morphological and structural similarities between other organs of these species, especially regarding the placenta (Bosco, 1997; Bosco et al., 2007; Mess et al., 2007; Valdés et al., 2008).

The morphology, neurochemistry and electrical properties of guinea pig pancreatic neurons have been described by Liu and Kirchgessner (1997). Although their role in the physiology of exocrine and endocrine secretion is still under study, according to these authors pancreatic ganglia should not be regarded as a simple relay ganglia interposed between the vagus nerve and the effector organs. Indeed, the pancreatic ganglia are much more complex, and it is thought that because of this complexity, the pancreas displays a degree of independence when cut off from the brain, spinal cord, or gut (Stagner and Samols, 1985). Furthermore, the observation of spontaneous activity within connected pancreatic ganglia gives support to the idea of an endogenous neural network regulating pancreatic function (Liu and Kirchgessner, 1997).

The aim of this study was to acquire information regarding the morphology of ganglionar neurons in the degus pancreas in order to compare them to those described in the guinea pig (Kirchgessner and Pintar, 1991; Liu et al., 1996), to further our understanding of the function of this organ under normal and pathological conditions.

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http://dx.doi.org/10.1016/j.autneu.2013.05.002

2. Materials and Methods

A colony of 10 adult male *O. degus*, weighing 199 ± 15 g, bred in the animal facility at the Department of Anatomy and Developmental Biology, was used in this study. The animals received food and water ad libitum. The handling of the degus was carried out according to internationally accepted ethical standards, after approval of the Animal Care Committee of the Faculty of Medicine, University of Chile. The animals were slightly anesthetized with ether (Merck, Darmstadt, Germany) and subsequently sacrificed by overdose of sodium pentobarbital (80 mg/kg i.p). For optical microscopy studies, the pancreas of each animal was dissected and fixed for 24 h by immersion in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.3), embedded in paraffin wax, and cut into 5 μm sections. Routine histological analysis was performed using the hematoxylin–eosin (H/E) technique.

2.1. Immunohistochemical procedures

Standard immunoperoxidase procedure was used to evidence the ganglionar neurons in the pancreas, as well as the distribution of somatostatin positive cells in the islets of Langerhans. Briefly, mouse anti-human neuron specific enolase (NSE) monoclonal antibody (M0873 DAKO, USA), diluted 1:100 (v/v) was applied to the tissue sections for 30 min at 37 °C. In order to obtain optimal staining, microwave heat-induced antigen retrieval in citrate buffer, pH 6.0, was performed. In many of the pancreas samples the same transversal duodenum section appears allowing us to identify the neurons of the submucosal neural plexus and the myenteric plexus of the muscular layer using immunohistochemistry Rabbit anti-human polyclonal somatostatin (M0762 DAKO, USA), diluted 1:200 (v/v) was applied individually to each section for 30 min at 37 °C. Antibodies were omitted in negative controls for NSE and somatostatin. Immunostaining was revealed using the horseradish peroxidase-labelled streptavidin biotin kit (DAKO, USA), according to the manufacturer's directions, using 3, 3′diaminobenzidine (DAB) as chromogen. All sections were counterstained with Mayer's hematoxylin (DAKO, USA), mounted using Entellan (Merck) and examined by light microscopy (Zeiss Axioplan 2, Germany).

2.2. Electron microscopy procedures

The samples were fixed in 3.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH, 7.3) at 4 °C for 3 h and subsequently postfixed for 1 h in 2% osmium tetroxide, prepared in the same buffer. The fixed samples were then dehydrated in ascending grades of ethanol, cleared in propylene oxide and embedded in Epon 812 (EMS, USA). Sections were cut in an OM-U2 ultramicrotome (Richert, Germany) and were stained with uranyl acetate and lead citrate prior to examination under an electron microscope (EM 109; Zeiss; Güttingen, Germany). For orientation purposes, semi-thin sections were stained with 1% toluidine blue in 1% sodium tetraborate and were examined by light microscopy.

3. Results

The pancreas of the degus is enveloped by a thin capsule of connective tissue from which septa extend into the organ, thus separating the pancreatic lobules. As in all animals, this organ is a mixed of exocrine and endocrine glands, constituted by acini and islets of Langerhans (Fig. 1A). The acinar cells display the characteristic serous type of a protein-synthesizing cell: round nuclei located in the basal third (see inset in Fig. 1A). The secretory granules accumulated in the supranuclear region are strongly stained with H/E (Fig. 1A and C). The average size of the islets of Langerhans is 79 ± 15 μm in diameter. Some groups of pancreatic ganglionar neurons (GN) were observed at optical microscopy level using H/E (Fig. 1C and D) and confirmed by positive NSE immunostaining (Fig. 2C, D and E). The pancreatic GN showed a spherical, large and faintly stained nucleus displaying a prominent nucleolus. As specified by the manufacturing company, NSE also recognizes neuroendocrine cells of the islets of Langerhans (Fig. 2C and D). Each pancreatic GN is surrounded by satellite glial cells, smaller than the neurones and displaying a chromatinic nucleus (see Fig. 1D). We have included in this study a photomicrograph of degus diabetic islets of Langerhans (Fig. 1B) and of the duodenal enteric nervous system (Fig. 2A and B) to emphasize the differences and similarities observed between these structures and the pancreatic GN.

Somatostatin secreting delta cells (D cells) in the islets of Langerhans were identified by positive cytoplasmic immunostaining with rabbit anti human polyclonal somatostatin antibody (see Fig. 3A, B and D). This antibody rendered negative immunostaining in pancreatic GN (Fig. 3A, C and D).

Electron microscopy evidenced the presence of some unmyelinated axons in the interlobular spaces or septas, usually located adjacent to blood vessels and the pancreatic exocrine epithelial ducts and in proximity to lymphatic capillaries (Fig. 4), in agreement with a previous report (O'Morchoe, 1997). In the interlobular space it was possible to observe the presence of telocytes with at least two telopodes, frequently establishing a close spatial relationship with blood vessels (Fig. 4) and nerve endings (Fig. 5). Telocyte location seemed to follow a pattern, being often observed in the vicinity or in close contact with both secretory (acini and exocrine epithelia ducts) and regulatory apparatuses (nerves and blood vessels) (see Figs. 4 and 5), as it has been recently described (Nicolescu and Popescu, 2012).

4. Discussion

As early as 1869, Langerhans observed that pancreatic islets were innervated. Since then, neural elements in the various compartments of the mammalian pancreas have been extensively studied at anatomical, neurochemical and functional levels Wang et al. (1999b). To our knowledge, this is the first time that the presence of neuronal cells is described in the pancreas of the *O. degus*.

Neurogastroenterology is defined as neurology of the gastrointestinal tract, liver, gallbladder and pancreas and it encompasses control of the digestive process through the enteric nervous system (ENS) (Kiba, 2004), the central nervous system (CNS) and integrative centers in sympathetic ganglia. Neurons in the ENS pathways are related to a wide range of chemical messengers that signal through an even wider range of receptors, which may provide potential targets, playing some role in the modification of digestive functions such as motility, secretion and blood flow (Furness, 2012; Roberts et al., 2010).

ENS was a concept introduced in order to characterize the peculiarity of the neuronal elements observed within the gut wall (Langley, 1900). ENS is a component of the neural control system of the digestive tract, working in concert with the CNS integrative pathways that pass through sympathetic ganglia and the gastroenteropancreatic endocrine system (Furness, 2012). Many functions of the digestive system as well as some functions related to digestion, such as satiety, involve both enteric innervation and the endocrine system of the digestive tract or gastroenteropancreatic endocrine system. In fact, most aspects of gastrointestinal control involve both neurons and endocrine cells. We postulate that the degus' pancreatic neuronal cells probably belong to the ENS and may share the same or similar characteristics to those reported in the pancreas of the guinea pig (Liu and Kirchgessner, 1997), where its influence upon the activity of the organ has been thoroughly described.

Studies in the pancreatic ganglia of the guinea pig have shown that the organ is innervated by neurons originated from neural precursor cells located in the wall of the gut, that migrate into the pancreas from the bowel (Kirchgessner et al., 1994). Hence, it has been concluded that the innervation of the pancreas may be considered as an extension of the ENS. Furthermore, the vagal neural crest is considered to be the embryological origin of most of the neurons and glial cells that constitute the ENS (Burns et al., 2000; Kirchgessner et al., 1994; Young and