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Research Article

Parietal and Enteroendocrine Cells from the Degu (*Octodon Degus*) Stomach Glands: An Immunohistochemical and Ultrastructural Study

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Abstract

The 'degu' is a caviomorph rodent, active mainly at daytime and feeding fundamentally on plants. It is endemic to Chile, inhabiting areas of low water availability. The present study aimed to analyze, at optical and electron microscopy levels, the morphological characteristics of parietal cells in the degu's stomach, located in the fundus and body regions of this organ. These cells require an adequate water balance in order to produce HCl involved in the maintenance of the pH for the process of protein digestion. A complementary experimental approach using immunohistochemistry for somatostatin and serotonin was also followed. Somatostatin is an inhibitor of HCl production, and serotonin is responsible for the digestive system peristaltic movements. The gastric glands in the fundus and body regions of the degu's stomach appeared shorter than those observed in the human's stomach, displaying abundant parietal cells interspersed with numerous serotonin positive enter endocrine cells. In the antral zone of the gastric mucosa, only a few somatostatin positive enter endocrine cells were observed at the bottom of these glands. Ultra structurally, parietal cells at rest displayed abundant mitochondria and cyoplasmic tubular-membrane vesicles. Following stimulation, this tubular-membrane vesicles system rearranges itself in order to form microvilli and expanded canaliculi in the cellular apical region. We conclude that the degu's parietal cells show similar structural characteristics to those of their human counterparts, and that they are probably paracrinally inhibited by the somatostatin secreted in the antral glands. Taking into consideration that the degu is an herbivorous animals with a diet containing a considerable amount of fibres, its digestive system probably requires strong and powerful peristaltic movements, which may be regulated by the serotonin produced in the gastric glands cells.

Keywords: *Degu; Parietal cells; Enteroendocrine cells; Stomach glands*

Introduction

The degu (*Octodon degus*) is an herbivorous [1,2] hystricomorph, caviomorph rodent, active mainly in daytime [3] that lives in colony groups composed of 5 to 10 animals [4]. It is native to Chile and inhabits the semiarid Northern regions of the country, where water is scarce [3,5,6]. In this context, the study of Cortés *et al.* [5] demonstrated that this animal is capable of surviving without liquid intake for 13.4 days. This fact indicates that their survival depends on both physiological and morphological adaptations for water saving, i.e., low water loss by evaporation, high condensation of water through the nasal spaces, urinary concentration and fecal dehydration [6]. It is worth noting that these adaptations favor the maintenance of the cellular water balance, especially for those cells whose functional metabolism depends directly on this element. The stomach, in particular, is the organ where the parietal cells, located in the fundus and body, physiologically produce HCl. This acid is produced through a series of metabolic processes in which the cellular water balance is critically maintained by means of osmotic diffusion of water into the cytoplasm [7].

Taking these considerations into account, we considered the study of the morphological and structural characteristics of the parietal cells in the degu stomach to be of importance, as no information regarding this organ of the digestive system of this species has been reported so far. The only example in the literature regarding this topic is the study of the degu's colon by Gallardo *et al.* [6], who demonstrated that fecal dehydration in this rodent was achieved by means of transcellular water absorption via aquaporin (AQP) type AQP-1 channels, located in the epithelial lining of this organ. In addition, our group has recently demonstrated the presence of intra and extra pancreatic ganglionic neurons in the degu's pancreas, concomitant with the presence of telocytes in the same organ of the digestive system [8]. This latter type of cell has also been described in other human organs.

Therefore, the aim of the present study is to deepen and expand the information regarding the digestive system of this rodent. To this purpose, we performed a morphological ultrastructural analysis of the parietal cells in the gastric glands of the body and fundus of the degu's stomach, as well as an immunohistochemical study of the enteroendocrine cells that regulate the function of the parietal cells.

Material and Methods

A colony of 5 adult male *O degus,* weighing in average 190 ± 8g, 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,* and all experiments were performed according to international regulations for animal care and ethical standards approved by the Committee of Animal Bioethics of the Facultad de Medicina, Universidad de Chile.

The animals were sacrificed by an overdose of sodium pento-barbital (80 mg/kg i.p). An incision was made in the abdomen of each specimen and the stomach was exposed and dissected. For the histological and immunohistochemical procedures, the fundus, body and antral pylorus regions of the stomach were cut in half and the tissues were fixed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.3) for 24 h, embedded in paraffin wax and cut into 5 µm sections. Routine histological analysis was performed using the hematoxylin-eosin (H/E) technique to observe parietal cells in the fundus and body of the stomach. All sections were observed and photographed under a Zeiss Axiolab light microscope (Carl Zeiss, Germany).

Standard immunoperoxidase techniques were used to evidence somatostatin and serotonin immunohistochemical positive entero endocrine cells and to observe their distribution in the stomach glands. Rabbit anti-human somatostatin polyclonal antibody, diluted 1:200 (v/v) (A0566 DAKO), or mouse anti-human serotonin monoclonal antibody, diluted 1:50 (v/v) (M0758 DAKO) were individually applied to each section for 1 hr at 37°C. Immunostaining was performed using the horseradish peroxidase-labelled streptavidin biotin kit (DAKO) following the manufacturer's directions and using diaminobenzidine as the chromogen.

The histological sections were counterstained with Mayer's haematoxylin (DAKO) and mounted with Entellan (Merck). Immunohistochemical controls were done by replacing the primary antibodies with phosphate buffered saline. All controls were negative. All the sections were examined under light microscopy (Zeiss Axioplan 2).

For transmission electron microscopy, the samples were immersed in 3.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3, 4°C, for 3 h, washed in the same buffer and post-fixed for 1 h in 2% osmium tetroxide prepared in the same buffer. The samples were then dehydrated in ascending grades of ethanol, cleared in propylene oxide, and embedded in Araldite 502 [9]. For orientation purposes, semi-thin sections were collected and stained with 1% toluidine blue in 1% sodium tetraborate and examined under light microscopy. Ultra-thin sections were prepared using a Reichert OM-U2 ultra microtome and were contrasted with uranyl acetate and lead citrate [10], and examined and photographed in a JEOL/JEM 100 SX electron microscope.

Results

The fundus and body of the degu's stomach are lined by an epithelium of mucus-secreting columnar cells (Figure 1A), with the presence of fundic glands in the lamina propria of the mucosa layer (Figure 1A). These glands show numerous parietal cells with an ovoid cell body of 16µm diameter and displaying a central round nucleus. The cytoplasm displays a strongly acidophilic reaction to the H/E staining

(Figure 1A). These parietal cells are found throughout the entire gland, except in its bottom part, where zymogen-producing cells, the main producers of pepsinogen are located. In contrast to the parietal cells, the zymogen cells evidenced a weak basophil cytoplasm (Figure 1A). Enteroendocrine cells are not observed under H/E staining.

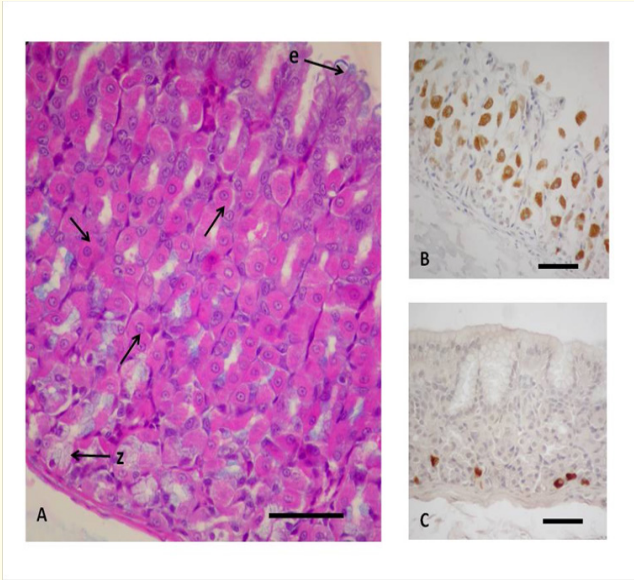

Figure 1: Gastric and antral glands of the degu's mucosa. A) Longitudinal section of a degu stomach, depicting the glands of the gastric mucosa at the fundus region of the organ. The lumen (right upper corner) is linned by a cylindric simple epithelium (e). The lamina propia shows the presence of gastric glands with an abundance of parietal cells (arrows) distributed along the entire gland, with the exception of the bottom region, where the zimogen cells (z) are predominantly located. H/E staining. B) Serotonin positive cells intermingled with the parietal and epitelial cells from the gastric gland. C) Antral glands showing the presence of a few somatostatin positive cells located at the bottom region of these glands.

Calibration bars: 48 µm in all figures.

Immunohistochemistry for serotonin yielded a strong positive reaction in a considerable number of polygonal cells, whose cell bodies are bigger than those that are somatostatin positive. These serotonin positive cells were exclusively located in the fundic glands (Figure 1B). On the contrary, immunohistochemistry for somatostatin evidenced only a few strong positive cells, located exclusively in the bottom region of the antral glands (Figure 1C).

Our EM study shows that the cytoplasm of parietal cells at rest evidences a great number of tubular-membrane vesicles as well as abundant mitochondria (Figure 2A). The intra-cytoplasmic disposition of the cell membrane that constitute the canaliculi [11], is not evident in this image, in accordance with the fact that these subcellular structures are collapsed in parietal cells in state of rest (Figure 2A). Under stimulation, the tubular-membrane vesicles are less evident, contrasting with a massive expansion of the canaliculi surface, produced at the expense of the formation of abundant microvilli toward the expanded canaliculi (Figure 2B) in continuity with the lumen of the fundic gland. A capillary blood vessel located beneath the basal region of a parietal cell at rest is also shown in Figure 2C.

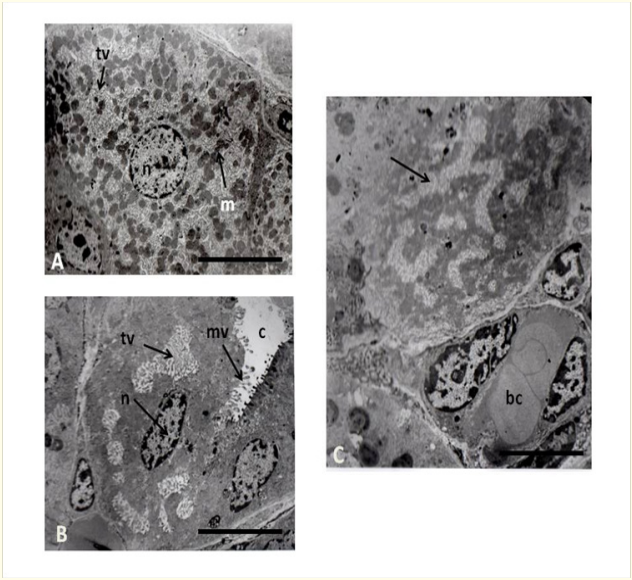

Figure 2: *Transmission electron microphotography of the degu stomach parietal cells. A) Parietal cell at state of rest showing the cytoplasmic membranous tubulo-vesicles (tv), mitocondria (m) and its centrally located nucleus (n). B) Active parietal cell evidencing scarse membranous tubulo-vesicles (tv) and abundant microvilli (mv) converging toward the canaliculi (c). C) Another resting parietal cell displaying numerous cytoplasmic tubulo-vesicles (tv) and mitochondria (m). At the basal región of the cell a blood capillar (bc) vessel can be observed. Calibration bars: 5 µm in all figures.*

Discussion

The evidence provided by our optical microscopy study allows us to state that the parietal HCl producing cells are located in the fundic glands of the fundus and body of the degu stomach. This finding is in accordance with the distribution of these cells reported in the human oxyntic glands [12]. The parietal cells in degu are very abundant and distributed along the entire gland (Figure 1A), with the exception of the bottom region, where zymogen cells, the main producer of pepsinogen, are observed.

Interestingly, in the human stomach these glands are comparative longer, due in part to the presence of a greater number of zymogen cells [12]. We postulate here that this difference is probably a consequence of the degu's diet, which is mainly based on fibrous plants with a moderate to low presence of nonstructural carbohydrates [13] such as sugar and starch. It is also worth noting that the degu´s gastrointestinal tract is structurally adapted to promote symbiotic microbial digestion of vegetable fibers such as cellulose and hemicelluloses (fibrous or structural carbohydrates). Therefore, a high intake of nonstructural carbohydrates will affect the intestinal microbial flora and consequently, the animal will be prone to developing diseases such as obesity and diabetes mellitus [2].

In general, the strong acidophilia displayed by the parietal cell in the fundic glands is due to an abundance of mitochondria [12], cellular organelles necessary to provide energy (ATP) for the secretion of HCl [14]. HCl production strongly contributes to maintaining the acidic pH in the stomach, necessary for protein digestion, as well as the absorption of iron, calcium and vitamin B12. This acidic pH also acts as a protection against exogen microorganisms [15].

Regarding the ultrastructure of the degu's parietal cells at rest, our results are in agreement with ME studies in the human stomach [14] and the rabbit stomach [16] that showed that the parietal cells in these species evidenced a considerable number of membranous tubule-vesicles and also abundant mitochondria. Forte *et al.* [17,18] demonstrated that the enzyme H+/K+-ATPase, of great importance in the HCl synthesis, was located in these tubule-vesicles. When the parietal cells are stimulated, the membranous tubulo-vesicles are fused into the apical domain of the cytoplasmic membrane in order to increase the surface of the cell. This is achieved through the formation of microvilli and the expansion of the canaliculi (Figure 2B), which allows the proper disposition of the enzyme H^+ pump. This subcellular restructuration undergone by the active cells is regulated by actin microfilaments and other cytoskeletal proteins [17]. Accordingly, the stimulation of the parietal cells in the gastric glands also produces a massive transformation of membranes, changing the position of H^+ pumps from the cytoplasmic tubule-vesicles domain into the microvilli at the apical plasma membrane domain. It is worth noting that Figure 2B in this work clearly shows the disposition adopted by the microvilli and canaliculi, confirming the active status of the cell [11]. As it was mentioned before, the presence of membranous tubule-vesicles concomitantly with the lack of microvilli and expanded canaliculi in the cells on Figure 2A and 2C, support the notion that those cells are in a state of rest. Figure 2C also shows a capillary blood vessel located in the vicinity of the basal zone of parietal cell in state of rest. Its function is related with the contribution of the Cl- ions required for the extracellular production of HCl, as well as the CO_2 necessary for the production of $-HCO_3$, by the action of the enzyme carbonic anhydrase [7], (Figure 3).

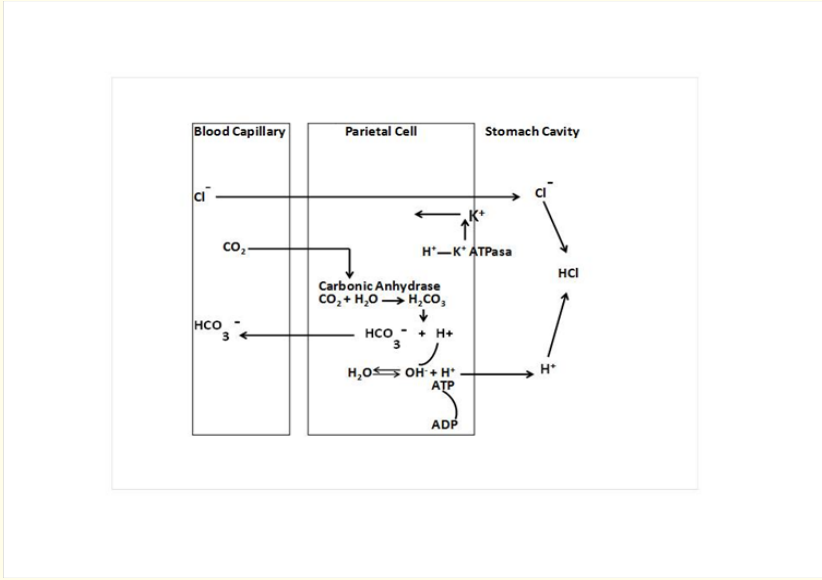

Figure 3: *Classic schematic representation of the HCl synthesis at the stomach parietal cell. Cloruro ions (Cl-) is actively transported from the capillar into the stomach cavity through the canaliculi. Water dissociates into hydrogen and hydroxyl ions in the parietal cell cytoplasm and hydrogen ions are actively transported into the canaliculi in exchange for potassium ions through the H-K-ATP-ase. HCl is consequently produced and secreted to the gland lumen through the canaliculi. Carbone dioxide, synthetized by cellular metabolism or from the blood circulation, reacts with water under the influence of the carbonic anhydrase enzime, forming bicarbonate ions, which diffuse into the blood in exchange for chloride ions, and the water is restituted.*

Our results indicate that the degu's parietal cells display the same morphological characteristics as the human parietal cells, differing only in that they are more abundant in the glands of the body and fundus of the animal stomach. This analysis leads us to postulate that the physiology of these cells would be quite similar in both species.

Figure 3 summarizes the classical pathway for the production of HCl, where it is worth noting that the water balance, both in the intra and extra cytoplasmic compartment, is central to the function of these parietal cells. Hence, the acquoporine channels in the epithelium lining the colon [6] play an important physiological role in the maintenance of this equilibrium. The gastric secretion of HCl is regulated by the interaction of endocrine, paracrine and neurocrine signals. This secretion is stimulated by histamine, gastrin and acetylcholine, and it is inhibited by somatostatin [15].

Furthermore, in the gastric mucosa glands of the degu's stomach (Figure 1B and C), our immunohistochemical analysis evidenced the presence of somatostatin (Figure 1C) and serotonin (Figure 1B) positive cells. The regional distribution of these enteroendocrine cells in the degu's stomach coincides with the region where these cells are located in the human stomach glands [12], the only difference being that the human mucosa layer presents deeper foveolae than those observed in the antral area in the degu stomach.

Importantly, somatostatin (produced by D-cells) inhibits the function of parietal cells [19] inhibiting in a paracrine way the gastrin secretion produced by G-cells located in the antral area [20]. As a consequence, this also inhibits the HCl secretion. The presence of H^+ ions in the gland's lumen constitutes the stimulus that activates D-cells, and thus, helps by means of a feedback mechanism, the inhibition of HCl production [21]. However, some drugs that inhibit HCl production act by binding covalently to the H^+ K (+)-ATPase, the hydrogen pump of the parietal cells, thereby irreversibly blocking acid secretion in response to all modes of stimulation [21].

On the other hand, Tzaneva [22] and Penkova *et al.* [23] demonstrated the existence of enter endocrine cells in the human gastric mucosa that produce serotonin. This hormone, which is also a neurotransmitter, plays an important role in the regulation of gastric motility, secretion and pain perception of the gastrointestinal tract and has also been associated with symptoms of gastrointestinal disorders such as nausea, vomiting, stomach pain, diarrhea and constipation. These authors found these cells in the epithelium as well as in the glands located in the fundus of the human stomach, in a similar way to what has been described in the present work for the degu (Figure 1C). These latter findings are also in agreement with those reported by Glisic *et al.* [24] on the rat stomach.

Regarding the large number of the serotonin producing cells in the degu's fundic glands, the results reported here are in agreement with Ceccarelli *et al.* [25] who reported the observation of a high number of the same enteroendocrine cells in the horse stomach, another herviborous animal; although in this species they are abundantly located in the antral region. Notwithstanding, these observations allow us to postulate that herbivorous animals present, either in the fundus or the antral region of their stomachs a high number of serotonin-producing cells in order to strengthen the organ peristalsis [26].

Summarizing, we concluded that in the degu stomach there are a considerable number of parietal cells along the whole extension of the fundic glands located in the fundus and body regions of the organ, concomitant with abundant serotonin producing enter endocrine cells. On the contrary, somatostatin secreting cells were restricted to the bottom of the antral glands.

Acknowledgmets

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