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Ultrastructural investigation on bovine Brunner's glands

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ABSTRACT

Brunner's glands are branched tubulo-acinar glands principally located within the lamina propria and the submucosa of the small intestine of mammals. Previous histochemical studies of Brunner's glands in cattle have shown that the glandular lobules can be divided into two parts: a peripheral part, corresponding to the terminal tracts of the gland, and a central part, corresponding to the pre-terminal tracts and to secretory duct of the gland. Aim of our study was to perform an ultrastructural investigation to verify whether the histochemical partition of the gland corresponded to the presence of different cell types, as described in other species. Data demonstrated that the glands were formed by two cell types: the tubulo-acinar gland regions consisted of pyramidal cells with large electron-lucent mucous secretory vesicles, while the pre-terminal and secretory ducts were made of sero-mucous cells with variable number of discrete secretory vesicles of different electron-density.

1. Introduction

Brunner's glands are branched tubulo-acinar glands, unique to mammalian species, mainly located within the lamina propria and the submucosa of proximal small intestine [4,9]. Generally they begin at the pyloro-duodenal junction and extend for a variable distance distally in the proximal small intestine; the position and the extension of Brunner's glands are reported to be variable according to the different groups of mammals. In man, these glands are localized in the mucosal lamina propria of the proximal duodenum but also invade the submucosa that distally becomes the only localization for these glands. In many mammals (i.e., cattle, horse, pig) Brunner's glands also extend to the jejunum for a variable distance. They are also reported as duodenal glands or submucosal glands on account of their extension and position, but the name of Brunner's glands proposed by Ref. [16] appears to be the most appropriate. The glands consist of several independent lobular masses, well delimited by connective tissue of submucosa or of mucosal lamina propria. In each glandular lobule, several secretory units are drained by an intralobular duct that reaches the bases of the duodenal crypts or the intestinal lumen and empties between villi. The secretory unit primarily consists of cells, which are described as typical mucous cells in humans [15]. In some animals, the cells are reported to exhibit the fine structural features of both serous and mucous-secreting cells, as in cats [17], mice [8] and pigs [20]. In rabbits, horses and ponies two cell types were contemporary observed. In horses serous cells were localized in the blind ends of the secretory tubules in the proximal 10 cm of the duodenum while the remaining part of the gland was made of mucous cells [14,19]. In cattle Brunner's glands are reported to be made exclusively of mucous cells [3,11,21].

The primary function of Brunner's glands is to produce a highly viscous mucus-rich alkaline secretion to protect the duodenal mucosa from the effects of acids, pepsin and other damaging agents [7,12]. Duodenal acidification elicits an increase in Brunner's glands secretion. The glands' response occurs in two phases, the first motor-dependent and the second mediated by local and nonlocal factors [13]. [1] have shown that duodenal mucosal bicarbonate secretion is independent of Brunner's glands in rats and rabbits.

The histochemistry of Brunner's glands secretion highlighted that mucins are mostly composed of neutral glycoproteins, whereas acidic glycoproteins have also been described in some species, herbivores principally [11]. Glycoproteins are characterized by peptide sequences rich in serine, threonine, and proline which carry large numbers of *O*-linked oligosaccharide chains which are specific and are considered to play critical roles in protecting mucous membrane [5,6]. In cattle histochemical studies pointed out that tubular and/or acinar blind ends are typically located in the peripheral portion of the lobule, and are involved in the secretion of N-glycoside-linked glycoconjugates. The branched secretory tubules opening in the secretory duct, and well as secretory duct itself, are both located in the central portion of the lobule, and elaborate mainly O-glycoside-linked glycoconjugates [23].

The identification of numerous additional factors (such as epidermal growth factor/urogastrone, IGF-I receptors, pepsinogens,

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prostaglandin) and enzymes (i.e., carbonic anhydrase, lysozyma, lipase) in the Brunner's glands of some species [11] highlighted the complexity of the composition and functions of this secretion, which appears to provide both mucosal protection and digestive enzymes release. Duodenase, a potential activator of enteropeptidase, was isolated in bovine Brunner's glands [2,25].

In spite of these reports which indicate the heterogeneous composition of bovine Brunner's glands secretion, little information is available as regards their fine structure [24]. The main purpose of our study was to investigate the ultrastructural features of bovine Brunner's glands to verify the hypothesis that the reported histochemical partition of the gland [18,21,23] corresponds to the presence of different cell types, as described in other animals.

2. Material and methods

Samples of duodenal tissues about 50 cm caudal from the pylorus were taken, from ten 18-months old Charolaise and Limousine bulls in a slaughterhouse. The specimens were pre-fixed for 2 h at 4 °C in a solution containing 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 and then rinsed in the same buffer. The tissues were post-fixed in 1% osmium tetroxide for 2 h at room temperature, dehydrated trough a graded series of ethanol and embedded in Epon. Semithin and ultrathin sections were obtained with an ultratome ultracut-E (Reichert-Jung). Semithin sections were stained with toluidine blue 1% and sodium borate 1%, while ultra-thin sections were mounted on nickel grids, stained in uranyl acetate and subsequently in lead citrate, and finally observed with a Philips 208S TEM.

3. Results

In the blind ends or terminal tracts (T) of the glands the secretive epithelial cells were pyramidal in shape and measured approximately 7–8 µm × 12–15 µm (Figs. 1 and 2). The cells were in close lateral contact with the neighbouring cells by means of typical junctional complexes consisting of tight junction in the juxtaluminal surface, intermediate junctions and desmosomes in the deeper lateral surface (Figs. 2 and 3). The luminal surface was irregular due to the convex border and to the presence of a few short microvilli. The basement membrane appeared tightly adherent to the basal lamina. The cells had oval nucleus measuring 6–7 µm × 5.5 µm, with transversal long axis,

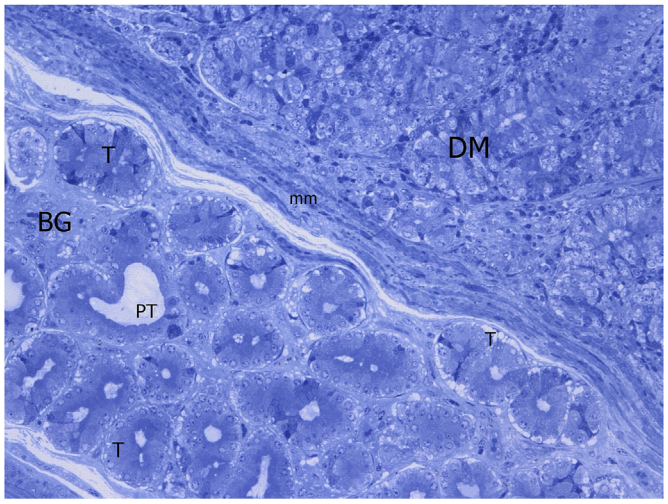

Fig. 1. Bovine duodenum with Brunner's glands. Toluidine blue. DM = duodenal mucosa; mm = *muscularis mucosae*; BG = Brunner's glands; T = terminal tract; PT = pre-terminal tract. X 20. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

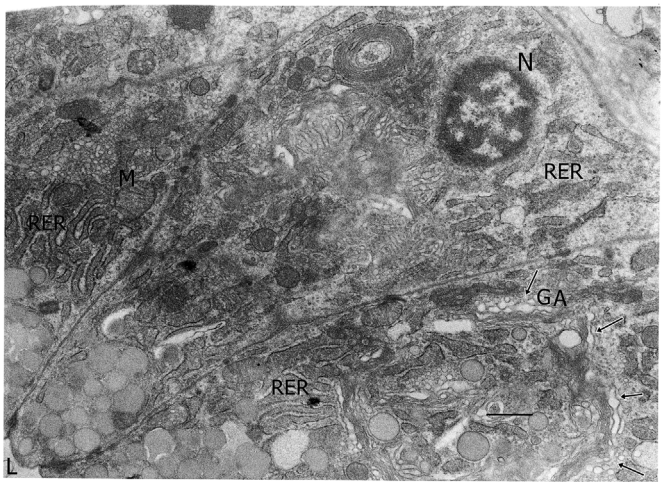

Fig. 2. Brunner's glands. Electron micrograph. Terminal tract (T). The nucleus is surrounded by rough endoplasmic reticulum tubules and cisternae. Multiple well-developed Golgi apparatus are observed. The stacks forming an ondulating line across the width of the cell are indicated (arrows). Mitochondria appear scattered in the whole cytoplasm but mostly concentrated in the basal and epinuclear regions. Osmium tetroxide fixation. N = nucleus; RER = rough endoplasmic reticulum; GA = Golgi apparatus; M = mitochondria; L = lumen. X 15,500 - marker 1 µm.

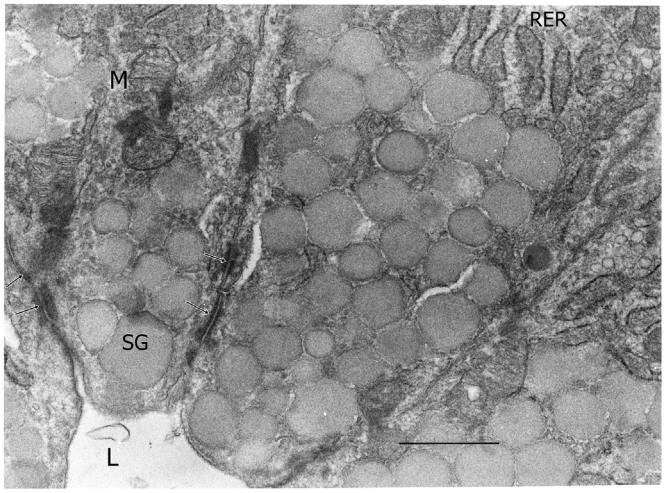

Fig. 3. Particular of the precedent micrograph. The secretive ephitelial cells facing the lumen are in close lateral contact by means of typical junctional complex (arrows). The apical part of the cell contains numerous electron-transparent vesicles. Some mitochondria are observed just below the secretory granules. Osmium tetroxide fixation. L = lumen; SG = secretory granules; M = mitochondria; RER = rough endoplasmic reticulum. X 33,750 - marker 1 µm.

located near the basement membrane, and provided with one of two nucleoli; irregular masses of heterochromatin were arranged at the periphery of the nucleus (Fig. 2). Inside the cell the exact localization of organelles suggested a definite polarity. The granular endoplasmic reticulum (RER), composed of tubules and cisternae containing an amorphous matrix, was mainly located in the perinuclear region, basally and laterally (Fig. 2); some cisternae were also observed in the supranuclear area between Golgi apparatus and secretory vesicles (Figs. 2 and 3). The cells contained multiple well-developed Golgi apparatus, mostly located in the epinuclear area and occupying an extensive region of the cytoplasm. The lamellae and cisternae of the Golgi complex appeared arranged in slightly curved stacks with convexity oriented towards the nucleus (Fig. 2). The stacks often formed an

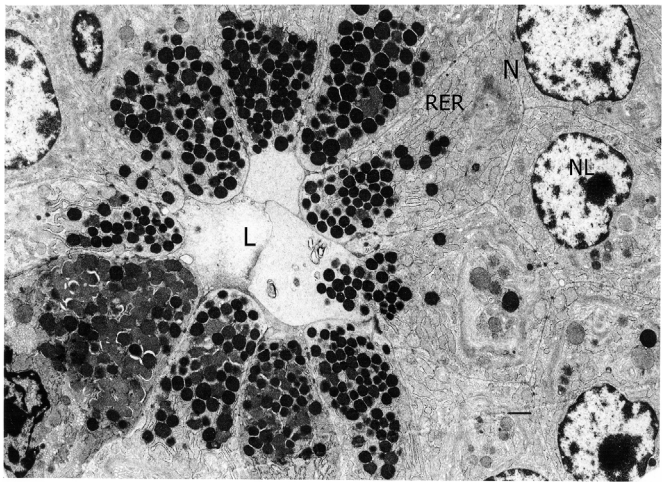

Fig. 4. Electron micrograph of the pre-terminal tract (PT). Glandular cells are arranged around a wide lumen. In the nucleus, nucleolus and peripheral condensation of heterochromatin are observed. Numerous electron dense secretory vesicles are located in the apical part of the cells. Osmium tetroxide fixation. L = lumen; N = nucleus; NL = nucleolus; RER = rough endoplasmic reticulum. X 7,000 - marker 1 μm.

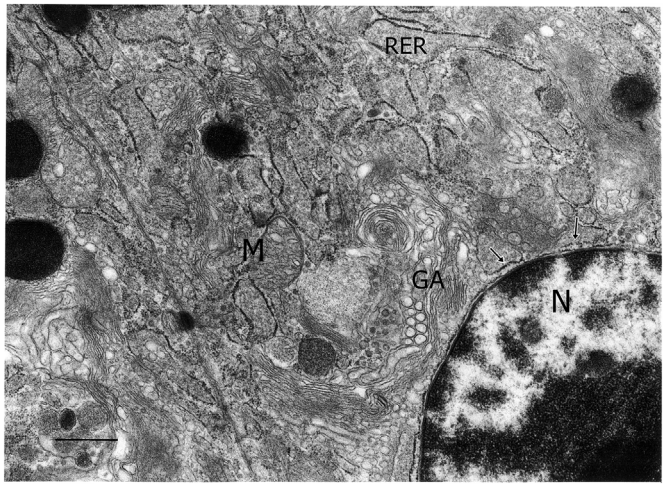

Fig. 5. Electron micrograph. Particular of the supranuclear cytoplasm of the pre-terminal tract (PT). The outer nuclear membrane continues with the membrane of the rough endoplasmic reticulum (arrows). Numerous mitochondria are scattered in the whole cytoplasm, some of them are noticed among the cisternae of RER. N = nucleus; RER = rough endoplasmic reticulum; GA = Golgi apparatus; M = mitochondria. Osmium tetroxide fixation. X 21,000 - marker 1 μm.

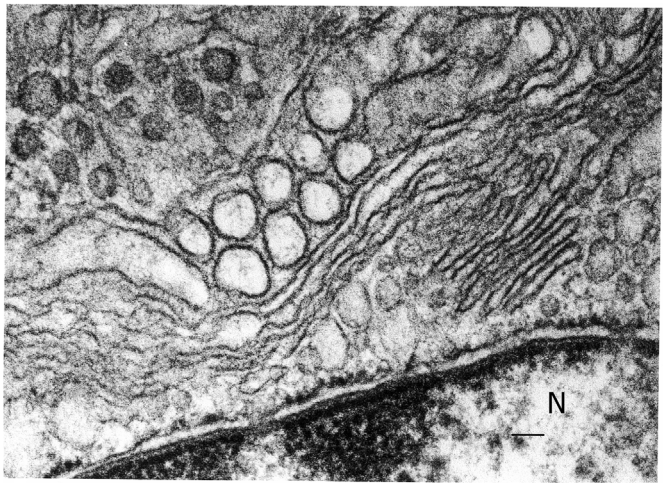

Fig. 6. Particular of the precedent micrograph. Section through the Golgi zone. The well-developed Golgi apparatus consists of numerous stacked flat sacs, large vacuoles and vesicles. Osmium tetroxide fixation. N = nucleus. X 105,000 - marker 100 nm.

undulating line running across the width of the cell (Fig. 2). Numerous transport vesicles and vacuoles of varying size were observed pinching off from periphery of Golgi lamellae on maturing face. In the apical part of the cells numerous big (0.7–2 μm in diameter) electron-transparent secretory vesicles were observed, often confluent to constitute compact masses pressing on the adluminal surface (Figs. 2 and 3). Mitochondria, ovoid or elongated, appeared scattered in the whole cytoplasm; they were most concentrated in the basal and epinuclear area whereas sometimes some of them were noticed among the secretory vesicles (Figs. 2 and 3).

In the branched pre-terminal tracts and in the secretory ducts (PT) the epithelial cells were from cubical to pyramidal in shape and measured approximately 7–8 μm × 9–12 μm (Figs. 1 and 4). The plasmatic membrane appeared similar to that of cells located in T on the lateral sides, while the luminal surface was not so convex as in T cells. These cells exhibited a round nucleus, basally located, that seemed to be medially smaller (6 μm × 5 μm) than the nucleus of T cells (Figs. 4 and 5). In the nucleus one or more nucleoli with associated heterochromatin were observed. The nucleus appeared encircled with some cisternae of the RER (Figs. 5 and 6). Numerous cisternae, whose surface was studded with ribosomes and polyribosomes, were otherwise located in the supranuclear region, between the well-developed Golgi apparatus and the apical area in which numerous secretory vesicles converged (Figs. 5 and 6). These cisternae were extremely dilated, filled with an amorphous matrix characterized by low electron-density (Figs. 5 and 6); some cisternae pushed through the secretory vesicles (Fig. 7). The apical cytoplasm belt appeared occupied by secretory vesicles with a variable electron-density and size (0.3–1 μm in diameter) (Fig. 4). Some of them appeared to be well-defined and with high electron-density, others exhibited not well-defined outlines and a lower electron-density. In the secretory vesicles characterized by low electron-density an electron-dense “core” was sometimes observed as reported in the pig by Ref. [22]. Numerous mitochondria were scattered in the whole cytoplasm, some of them were noticed among the dilated cisternae of RER (Fig. 7). In this part of the gland a few cells characterized by a dense cytoplasm and numerous vesicles were observed, probably corresponding to basal-granulated cells [10].

4. Discussion and conclusion

The fine structure of bovine Brunner glands outlined that they are made of two differently located cell types as indicated by histochemical analyse [18,21,23]. The cells located in T exhibited an extensive development of the Golgi apparatus in the supranuclear region, which underlines that these cells are mainly involved in the elaboration of the carbohydrate moiety of the glandular secretion. Differently the cells located in PT and in the secretory duct, characterized by numerous and often enlarged cisternae of RER, exhibited the fine structure of cells producing high quantity of both proteins and glycoproteins.

The previous classification which defined bovine Brunner's glands “mucous glands” is therefore non-exhaustive and neither effectively represents the nature and function of these glands, since only the blind ends turned out to be made of typical mucous cells, while the remaining part of the glands was formed by sero-mucous cells.

In truth the protein moiety function of the Brunner's glands is still not completely understood. The ultrastructural localization of duodenase in bovine Brunner's glands [26] revealed that it is mainly located in the secretory vesicles with low electron-density, i.e. in the rough

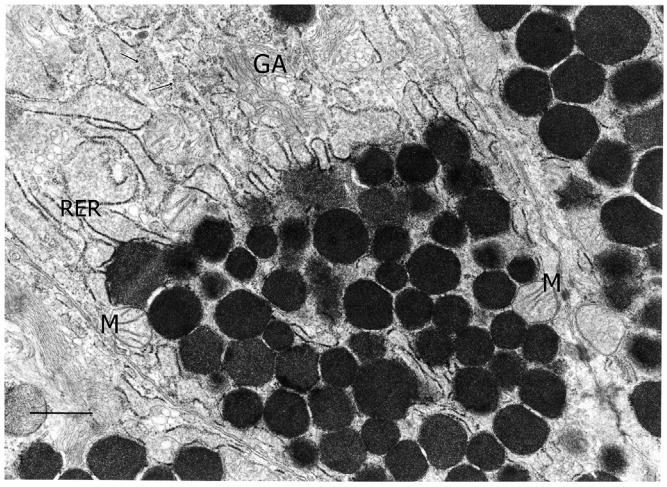

Fig. 7. Particular of the apical cytoplasm which appears occupied by secretory granules of varying electron-density and size. Some elongated cisternae of RER pushed through the secretive granules, as well as some mitochondria. Numerous polyribosomes (arrows) are scattered in the cytoplasm. Osmium tetroxide fixation. RER = rough endoplasmic reticulum; GA = Golgi apparatus; M = mitochondria. X 21,000 - marker 1 μm.

endoplasmic reticulum, the Golgi apparatus and in the secretory duct, presumably in the mucous cells, as photographic evidence. As a consequence, the epitheliocytes protein moiety of bovine Brunner's glands showing ultrastructural features mostly ascribable to protein secreting cells still remains unclear, since duodenase undoubtedly represents only a component of the protein moiety secreted by these glands. Further investigations are needed to characterize the protein moiety elaborated by bovine Brunner's glands; in addition, deepening this aspect would allow to ascertain the potential digestive and protection function of these glands.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tria.2019.02.001.

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