Journal of Advanced Veterinary Research

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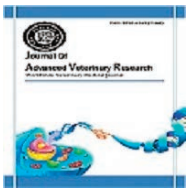

Assessment of the Endocrine Cells and Neural Structures in the Abomasum and Pancreas of Dromedary Camel based on their Synaptophysin Immunoreactivity

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ARTICLE INFO

Original Research

Received:
10 September 2020

Accepted:
06 October 2020

Keywords:

Abomasum, Camel, Immunohistochemistry, Pancreas, Synaptophysin

ABSTRACT

Endocrine cells of the gastrointestinal tract are located mainly within the pancreatic islets and throughout the wall of the stomach and intestines. These cells regulate several body functions via release of hormones. Synaptophysin is a transmembrane glycoprotein expressed in almost all types of endocrine cells as well as in synaptic vesicles of neurons. Nevertheless, the distribution of synaptophysin-immunoreactive (SYP-IR) cells in abomasum and pancreas of camel has not been described. In the present study, SYP immunoreaction was assessed in different regions of abomasum and pancreas of dromedary camel using SYP immunostained sections. SYP-IR endocrine cells of both closed- and open-types were observed within cardiac, fundic, and pyloric gland regions of the abomasal mucosa. Significantly higher number of SYP-IR cells were evident within the fundic and pyloric gland regions compared to cardiac gland region. Moreover, SYP labelled nerve fibers located within abomasal lamina propria and cells and fibers of the submucosal and myenteric nerve plexuses. In pancreas, SYP intensely labeled almost all cells of pancreatic islets. SYP-IR endocrine cells were also observed within the lining epithelium of pancreatic acini and ducts. In addition, SYP intensely stained cells and fibers of intrapancreatic ganglia. A moderate SYP immunoreaction was seen within the perivascular and periductal nerve fibers as well as those fibers supplying the pancreatic acini and ducts. These findings advance our understanding of the normal distribution of the gastro-pancreatic endocrine cells in camel. Future studies are needed for further characterization of hormones produced by these cells and their clinical relevance in camel.

J. Adv. Vet. Res. (2020), 10 (4),226-232

Introduction

The endocrine cells of the digestive system are distributed mainly in the form of clusters within the pancreas, the pancreatic islets, or scattered throughout the wall of the stomach and intestines, the gastric and enteric endocrine cells (Rindi *et al.*, 2004). Synaptophysin is an integral transmembrane glycoprotein expressed mainly in almost all types of endocrine cells (Wiedenmann *et al.*, 1986). It is also expressed in synaptic vesicles of neurons (Buffa *et al.*, 1987). Immunohistochemically, synaptophysin appeared as a regular constituent of the pancreatic endocrine cells in man, bovine, rodents, and dog (Redecker *et al.*, 1991). Such expression pattern renders synaptophysin as one of the most specific and consistent markers for identification of cells with neuroendocrine functions in normal and diseased tissues (Kyriakopoulos *et al.*, 2018). The pancreatic islets perform important roles for ensuring regular uptake of blood glucose through secretion of a cocktail of glucose-sensing hormones. Dysfunction of pancreatic islets is usually associated with nutrients deprivation in several tissues including liver, muscles, and adipose tissue (Nafikov and Beitz, 2007).

The endocrine cells in the wall of the gastrointestinal tract represent the largest population of endocrine cells in the body (Ahlman and Nilsson, 2001). Endocrine cells releasing several types of hormones were found across various regions of the stomach in mammals, birds, and reptiles (Aksoy and Cinar, 2009; Trandaburu and Trandaburu, 2009; Pyarokhil *et al.*, 2017). Through the release of multiple hormones and communications with the neural and vascular structures in the wall of the stomach, these gastric endocrine cells orchestrate body responses to various types of ingested nutrients and fine-tune a wide range of physiological processes (El-Salhy *et al.*, 2016). Thus, providing excellent therapeutic candidates for the treatment of diseases of endocrine origin.

Despite their importance for the food industry, several aspects of the gastric and pancreatic biology of camel are still poorly understood. Morphological, histochemical, and ultrastructural features of camel`s abomasal mucosa had been reported by previous studies (Abdel-Magied and Taha, 2003; Raji, 2011). The locations of cells expressing insulin, glucagon, somatostatin and pancreatic polypeptide in pancreatic islets of the dromedary camel had also been described (Althnaian

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ISSN: 2090-6277/2090-6269/

et al., 2019). However, data on quantitative distribution of endocrine cells in the abomasum and pancreas of camel are lacking. Taking the advantage of synaptophysin as a pan-endocrine marker, the present study aimed to examine the distribution of endocrine cells across different regions of the abomasum (cardia, fundus, and pylorus) as well as the pancreas (body, right and left lobes) in camel. The expression of synaptophysin by neural structures located within the abomasal wall and pancreas was also examined and recorded.

Materials and methods

Sample collection

Abomasa and pancreases of 10 male dromedary camels aged 2-15 years were sampled soon after their slaughter at Zagazig abattoir (Egypt) during January-April 2019. Tissue sampling was performed based on the known anatomy of the examined organs (Abdel-Magied and Taha, 2003; Masaad, 2007). All samples were labelled, fixed in 10% neutral buffered formalin, and processed for paraffin embedding. Next, sections of 4 µm thickness were cut, mounted on positively charged microscopic slides and allowed to dry.

Chemicals

All chemicals used in the present investigation were obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA), unless otherwise specified.

Immunohistochemistry

Paraffin sections were deparaffinized in xylene and rehydrated in descending grades of ethanol. The antigenic epitopes were retrieved by boiling the sections in 10 mM citrate buffer (pH 6.0) in microwave at 750 watts for 25 minutes. Endogenous peroxidase activity was blocked by incubating tissue sections in 0.3% hydrogen peroxide solution for 20 minutes at room temperature (RT). To block non-specific binding of the primary antibody, sections were washed in phosphate buffered saline and then incubated in 10% donkey serum for 1 hour at RT before application of the primary antibody. Next, blocked sections were incubated with monoclonal rabbit anti-synaptophysin (clone# EP158, ready-to-use, BioGenex, CA, USA) for 3 hours at RT. For negative control sections, the primary antibody was replaced with blocking buffer. All sections were incubated with biotinylated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, PA, USA) followed by VECTASTAIN Elite ABC kit (Vector laboratories, CA, USA), 30 minutes each. Bound antibodies were visualized by incubating the stained sections with diaminobenzidine solution (Sigma-Aldrich, MO, USA) for 5-10 minutes. All sections were counterstained with hematoxylin and photographed using Leica DM3000 light microscope.

Quantification of synaptophysin (SYP)-immunoreactions

Synaptophysin-immunoreactive (SYP-IR) endocrine cells within the abomasal mucosa and pancreas of camels were quantified using particle counting and analysis section of ImageJ Fiji software (Schneider *et al*., 2012). At least three different sections from each animal were analyzed per each part studied. The number of SYP-IR cells in the cardiac, fundic, and pyloric regions of the abomasum were counted and compared. For pancreas, the following parameters were analyzed across the body, right and left lobes of the pancreas: the percentage of area occupied by the SYP-IR cells relative to the total area of pancreas, the number of islets /1mm^2, the islet diameter (µm), the number of SYP-IR cells /islet, and the number of SYP-IR cells within pancreatic acini /1mm^2. The intensity of SYP staining (chromogens) in neural structures within the abomasal wall and pancreas of camel were calculated in ImageJ Fiji software using the method described by Crowe and Yue (2019).

Statistical analysis

Charts representing the distribution of SYP-IR cells within the abomasal mucosa of camel were designed using GraphPad Prism 7 (GraphPad Software, CA, USA). The differences between the selected regions of the studied organs were determined using one-way ANOVA followed by Tukey`s multiple comparison test. P-values ≤0.05 were considered significant.

Results

Distribution of synaptophysin-immunoreactive (SYP-IR) cells within the wall of the abomasum of the dromedary camel:

Synaptophysin intensely labeled the endocrine cells located within different regions of the abomasal mucosa (Figs. 1a-1i). The SYP-IR cells appeared having different shapes and displayed prominent cytoplasmic staining (Figs. 1b, 1c, 1e, 1f, 1h, 1i). In the cardiac gland region, the SYP-IR cells appeared with low density (16.0±3.0 cells /1mm^2) and were concentrated chiefly within the epithelial lining of the secretory acini of the basal parts of the mucus secreting cardiac glands (Fig. 1a). These cells were mainly of closed-type and appeared oval- or round-shaped (Figs. 1a, 1b, 1j). The open-type SYP-IR cells of the cardiac gland region appeared flask-shaped with their tapering ends showing less staining intensity and reaching the lumen of the acini (Fig. 1c). No SYP-IR cells were detected within the epithelium of the gastric pits (Fig. 1a).

Compared to those observed within the cardiac gland region, higher numbers of SYP-IR endocrine cells were observed within the fundic (141.0± 23.0 cells/1mm^2) and pyloric (122.0±34.0 cells/1mm^2) gland regions (Figs. 1d-1j). These cells appeared of both closed- and open-types (Figs. 1e, 1f). In the fundic gland region, the SYP-IR cells were mostly of closed type, though numerous open-type cells with elongated cytoplasmic processes were detected within the neck region of these glands (Figs. 1d, 1f). The SYP-IR cells located within the pyloric region displayed significant differences in their shapes and appeared mainly of open-type (Figs. 1g-1i). The latter type of cells were stellate- or spindle-shaped with their cytoplasmic processes travelling between other cells lining the acini to reach their lumens (Figs. 1h, 1i).

SYP also labelled the neural structures located within different layers of the abomasal wall. A moderate SYP immunoreaction was observed within the nerve fibers located within the lamina propria in between the secretory units of gastric glands (Fig. 2a) as well as fibers of the submucosal nerve plexus (Fig. 2b). As well, SYP strongly stained cells and fibers of the myenteric ganglia located within the tunica muscularis of camel`s abomasum (Figs. 2c, 2d).

Distribution of synaptophysin-immunoreactive (SYP-IR) cells within the pancreas of the dromedary camel

In pancreas, SYP-IR cells were observed chiefly within the pancreatic islets where it almost stained all islet cells, except those of islet microvasculature (Figs. 3a-3f). The islet SYP stained cells appeared with an intense cytoplasmic staining surrounding non-stained nuclei (Figs. 3d-3f). Cells with relatively darker SYP immunoreaction were noted to be present at the peripheral parts of the islets (Figs. 3d-f). SYP-IR cells of

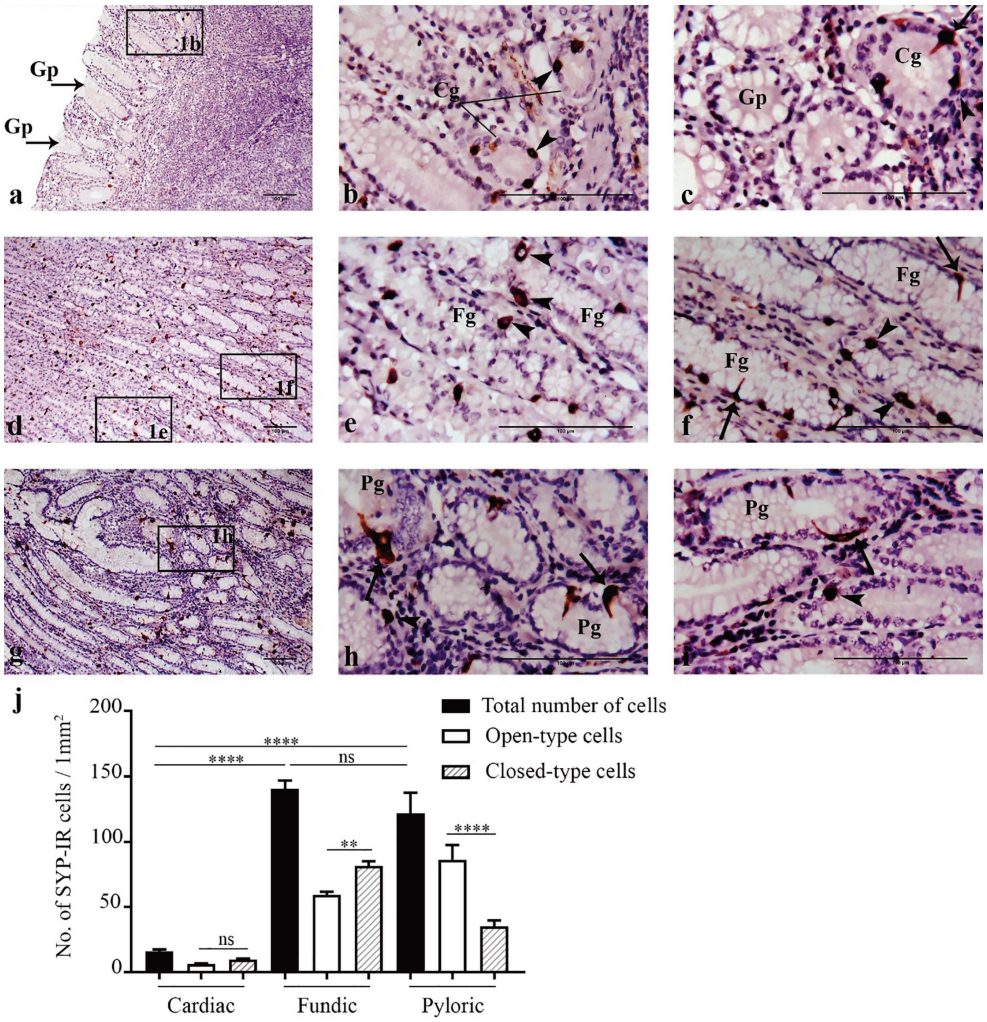

Fig. 1. Distribution of synaptophysin immunoreactive (SYP-IR) endocrine cells within different regions of the abomasal mucosa in dromedary camel. Representative photomicrographs of SYP-IR cells in the cardia (a-c), fundus (d-f), and pylorus (g-j) of camel`s abomasal mucosa. Figures 1b, 1e & 1f, and 1h are high magnifications of the areas marked by rectangles in figures 1a, 1d, and 1g respectively. Note the presence of closed- (arrowheads) and open-types (arrows) endocrine cells in all regions. Quantitative comparison of the distribution of SYP-IR cells within different regions of the abomasum in camel is shown in figure 1j. Significant differences are indicated by asterisks. ** = P < 0.01, **** = P < 0.0001, ns = not significant. Cg, cardiac glands; Fg, fundic glands; Gp, gastric pits; Pg, pyloric glands. Scale bar = 100 µm.

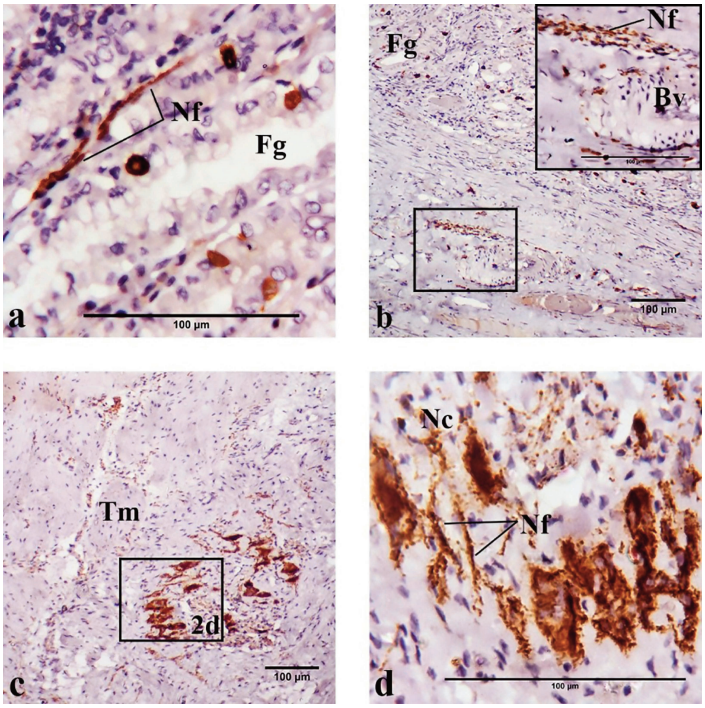

Fig. 2. Synaptophysin (SYP) immunoreactions within neural structures supplying the abomasal wall in dromedary camel. Representative photomicrographs of SYP immunoreaction in the nerve fibers (Nf) and cells (Nc) located within the lamina propria in between the secertory units of gastric glands (a), the sumucosal nerve plexus (b), and the myenteric ganglia (c, d). Figure 2d is a high magnifications of the area marked by square in figure 2c. Bv, blood vessel; Fg, fundic glands; Tm, tunica muscularis. Scale bar = 100 µm.

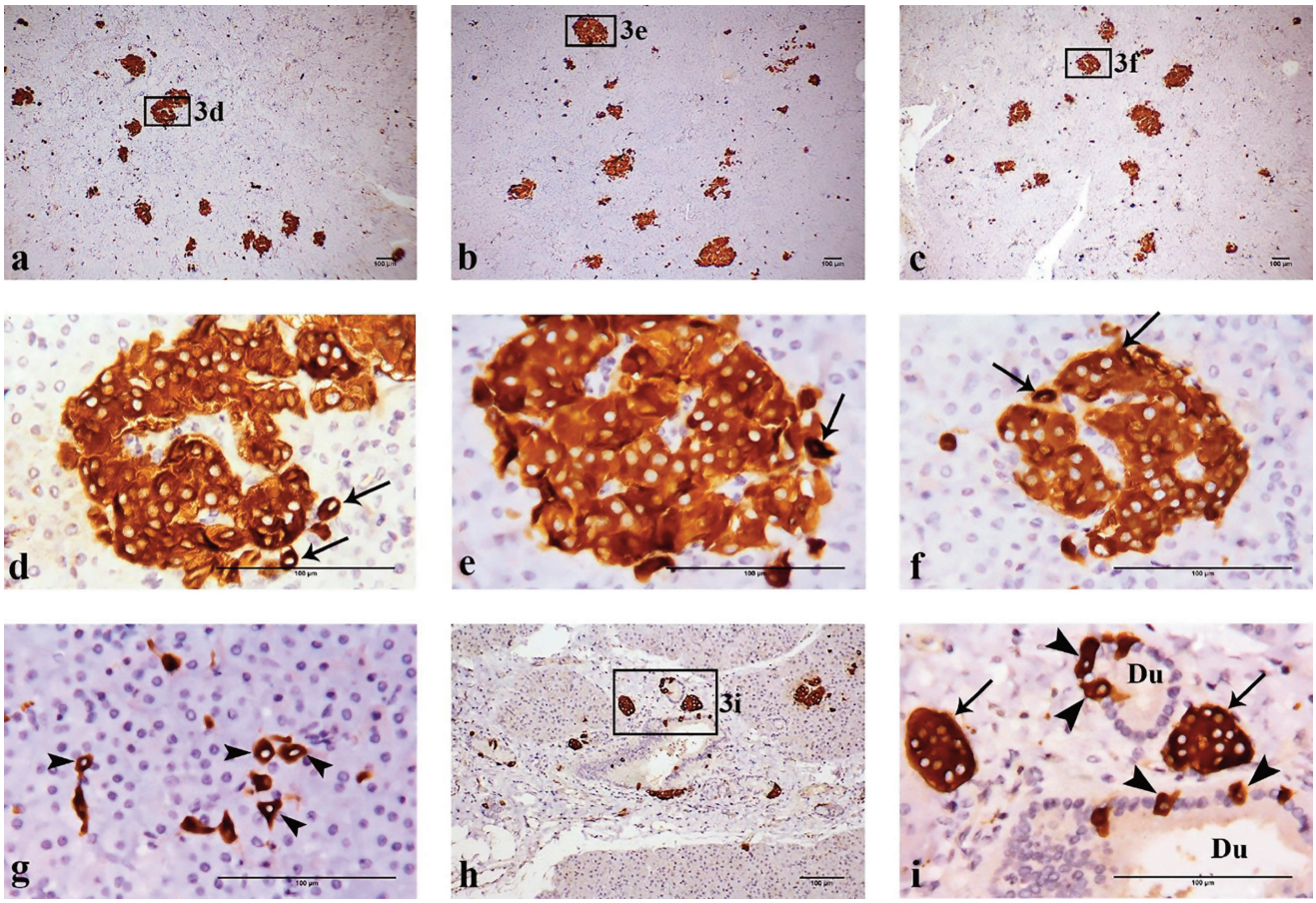

Fig. 3. Distribution of synaptophysin immunoreactive (SYP-IR) endocrine cells within different regions of the pancreas in dromedary camel. Representative photomicrographs of SYP-IR cells in the body (a, d), right lobe (b, e), and left lobe (c, f) of camel`s pancreas. Note the uniformity of the distribution of pancreatic islets within different parts of the pancreas (a-c). Cells with darker SYP immunostaining are occasionally seen at the peripheral parts of the islets in all regions of the pancreas (arrows in d-f). SYP-IR cells are also detected within the secretory acini of the exocrine pancreas (g, arrowheads) and the epithelial lining of pancreatic ducts (h, i; arrowheads in i). Small-sized SYP-IR pancreatic islets are constantly seen in the immediate vicinity of pancreatic ducts close to the ductal regions containing these SYP-IR cells (arrows in i). Figures 3d, 3e, 3f, and 3i are high magnifications of the areas marked by rectangles in figures 3a, 3b, 3c, and 3h respectively. Scale bar = 100 µm.

Table 1. Quantitative remarks on the distribution of synaptophysin immunoreactive (SYP-IR) endocrine cells within different parts of the pancreas of dromedary camel.

	Body	Right lobe	Left lobe
Area (%) occupied by SYP-IR endocrine cells relative to the total area of pancreas	2.5 ± 0.8^a	2.7 ± 0.9^a	2.6 ± 0.7^a
Number of SYP-IR islets / 1 mm^2	4.0 ± 0.8^a	4.0 ± 0.7^a	4.0 ± 0.8^a
Islet diameter (µm)	78.8 ± 26.7^a	78.5 ± 18.1^a	76.0 ± 17.8^a
Number of SYP-IR endocrine cells / 1 islet	122 ± 16^a	134.0 ± 25.0^a	108.0 ± 17.0^a
Number of SYP-IR endocrine cells within the acini of exocrine pancreas / 1 mm^2	4.0 ± 0.7^a	3.0 ± 0.9^a	4.0 ± 0.5^a

Superscript letters indicate significant differences between various regions of camel`s pancreas ($P \leq 0.05$).

different shapes were also found among the epithelial lining of the secretory acini of the exocrine pancreas (Fig. 3g).

SYP-IR cells of both closed- and open-types were detected among the epithelial lining of different ordered pancreatic ducts. These cells appeared with higher count in large pancreatic ducts (Figs. 3h, 3i). It is noteworthy that, small-sized SYP-IR pancreatic islets were constantly seen in the immediate vicinity of these intraductal SYP-IR cells (Figs. 3h, 3i).

The percentage of pancreatic area occupied by endocrine cells, including those present within the exocrine pancreas and ducts, was 2.6, the number of islets per every 1mm^2 of pancreas was 4, the average islet diameter (µm) was 78, the number of SYP-IR cells per islet was 121, and the number of SYP-IR cells located within pancreatic acini per each 1mm^2 of pancreas was 4 (Table 1). No significant difference was observed in any of these parameters between the body, left, and right lobes of the pancreas.

Regarding SYP expression in the neural structures of the pancreas, strong SYP immunoreaction was seen within the fibers and cells of the interlobular ganglia (Figs. 4a, 4b). Moderate SYP immunoreactions were also observed within the intrapancreatic periductal nerve fibers (Figs. 4c, 4d), perivascular nerve fibers (Figs. 4e, 4f), as well as the nerve fibers supplying the acini of the exocrine pancreas (Figs. 4f, 4g) and pancreatic islets (Fig. 4h).

Discussion

The present study reported for the first time the expression of synaptophysin, as a marker for neuroendocrine cells, within the wall of the abomasum and different parts of the pancreas in the dromedary camel. Synaptophysin revealed intense cytoplasmic staining in all cells of pancreatic islets, except those of islet blood vessels. These findings are consistent with the observations of Redecker *et al.* (1991) in pancreatic islets of rabbit, man, and dog. However, the results of the pres-

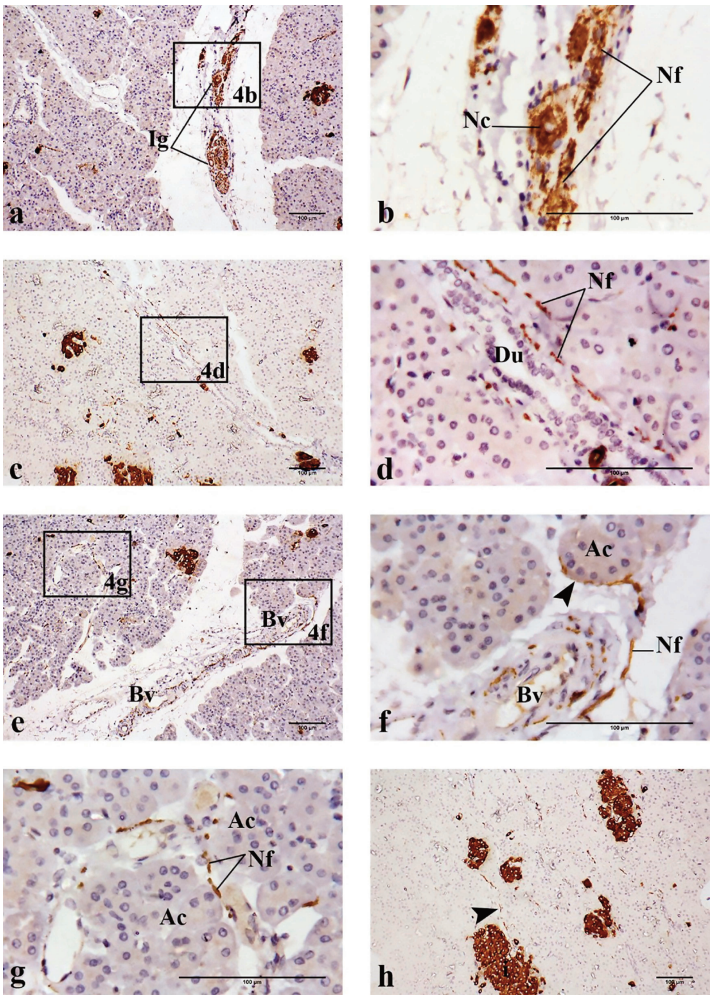

Fig. 4. Synaptophysin (SYP) immunoreactions within neural structures supplying the pancreas in dromedary camel. Representative photomicrographs of SYP immunoreaction in the nerve cells (Nc) and fibers (Nf) located within the interlobular pancreatic ganglia (a, b), the wall of pancreatic ducts (Du- c, d), the wall of pancreatic blood vessels (Bv- e, f) and the acini of the exocrine pancreas (Ac- e, g). Representative photomicrograph of SYP immunoreaction in the nerve fibers (arrowhead) supplying pancreatic islet (i) is shown in h. Figures 4b and 4f & 4g are high magnifications of the areas marked by rectangles in figures 4a and 4e respectively. Scale bar = 100 µm.

ent study are in disagreement with the findings of Wieczorek *et al*. (1998) in minipigs and non-human primates in which pancreatic islets displayed very weak immunostaining with synaptophysin antibody. Such discrepancy might be explained on the basis of the low sensitivity of antibody used by the latter authors. Moreover, the present study noted that cells at the periphery of the islets were having darker staining compared to cells in the center of the islets. The peripheral location of these darkly stained cells could suggest that these cells are glucagon and polypeptide producing cells (Althnaian *et al*., 2019). Thus, synaptophysin is possibly having a role in release of the latter mentioned hormones and the variation in its staining intensity among islet cells could be due to differences in rates of its storage and release between different types of islet cells. Similar enrichment of glucagon and polypeptide producing cells with synaptophysin staining was reported in pancreatic islets of mouse, rat, cow, and gerbil (Redecker *et al*., 1991).

In line with Merkwitz *et al*. (2013) in bovine pancreas, the present study observed the presence of synaptophysin positive endocrine cells within the lining epithelium of pancreatic ducts and the acini of the exocrine pancreas. The distribution of the endocrine cells within the three basic components of the pancreas, the pancreatic islets, ducts and secretory acini, are confirming the previous assumption indicating that these components are acting as a functionally integrated unit (Bertelli and Bendayan, 2005). The presence of numerous small-sized islets in the vicinity of pancreatic ducts at sites containing intraductal synaptophysin expressing cells are reinforcing previous studies suggesting ductal epithelium as a source for neogenesis of islets in adult mice, rat, and man (Park and Bendayan, 1994; Van de Casteele *et al*., 2013; Dirice *et al*., 2019). Despite the importance of these intraductal endocrine cells, these cells have not been reported by any of the previous immunohistochemical studies involving endocrine pancreas of camel (Hafez *et al*., 2015; Althnaian *et al*., 2019; Bargooth *et al*., 2020). The identity and nature of hormones produced by these intraductal synaptophysin expressing cells in camel`s pancreas warrant further investigation.

The morphometric analysis of pancreatic islets in camel`s pancreas included in the present study revealed that the mean islet diameter was 78 µm, and the density of islets in pancreas was 4 islets /mm^2. These values are almost similar to those reported in pancreas of normal human (78 µm and 4.4 islets /mm^2) subjects (Seiron *et al*., 2019), suggesting that both islet diameter and density might be evolutionary conserved among mammalian species.

The present study revealed the presence of synaptophysin immunolabelled cells within different regions of the abomasal mucosa in camel. This finding is in line with that of (Wiedenmann *et al*., 1988; Waldum *et al*., 2014) in human gastric mucosa. Portela-Gomes *et al*. (1999) observed stronger synaptophysin immunoreactivity within the human gastric mucosa compared to different intestinal segments. Moreover, the latter study noted colocalization of synaptophysin with several hormones, including gastrin, serotonin, and somatostatin, which might suggest a role of synaptophysin in transport and release of these hormones. In line with its important

role in functioning of gastric endocrine cells, synaptophysin positive endocrine cells were detected in the abomasum of developing goat by the 64th day of prenatal life (García *et al*., 2012). Numerous synaptophysin positive cells of open-type were observed within the lining epithelium of cardiac, fundic, and pyloric gland zones of the abomasum by the present study. The frequency of these cells were highest in the pyloric region. Unlike the closed-type endocrine cells, which are regulated mainly via neurohormonal signals, these open-type cells reach the lumen of the acini of the gastric glands and are more likely to be regulated directly by gastric contents (Solcia *et al*., 2000).

Synaptophysin immunopositive reactions were observed within the nerve fibers and cells located in the wall of the abomasum and in the pancreas of camel by the present work. Similar observations were reported in compound stomach of goat (García *et al*., 2012, 2014), stomach of rat (Asar *et al*., 2004), and gastro-pancreatic system of turtle (Trandaburu and Trandaburu, 2009). Such expression of synaptophysin in neural structures of these organs is in line with its role in conduction of nerve impulses (Valtorta *et al*., 2004).

Further studies are possibly needed to further characterize the identity of these cells in camel. Immunohistochemical techniques that allow for multiple labelling of these cells or their co-staining in serial sections will help for their further characterization. Hormones reported to be produced by pancreatic endocrine cells in other vertebrates include gastrin (Portela-Gomes *et al*., 1999), obestatin (Zhao *et al*., 2008), nucleobindin-2/nesfatin-1 and ghrelin (Stengel *et al*., 2013; Mohan *et al*., 2016). Glucagon, somatostatin, cholecystokinin-8, serotonin, secretin and histamine are hormones that have been shown to be secreted by gastric endocrine cells of other animals (Adnyane *et al*., 2011; Pyarokhil *et al*., 2017; Türk *et al*., 2019).

Conclusion

Synaptophysin-immunoreactive cells are present within the pancreatic islets, acini and ducts. These cells are also found throughout different regions of the abomasal mucosa, being highest within the fundic and pyloric regions. Overall, the results of the present study improve knowledge related to the distribution of gastropancreatic endocrine cells in camel and could form a basis for differentiation of diseases affecting them.

Conflict of interest

The author declares no conflict of interest.

References

Abdel-Magied, E.M., Taha, A.A.M., 2003. Morphological, morphometric and histochemical characterization of the gastric mucosa of the camel (*Camelus dromedarius*). Anatomia, Histologia, Embryologia 32, 42-47.

Adnyane, I.K.M., Zuki, A.B., Noordin, M.M., Agungpriyono, S., 2011. Immunohistochemical study of endocrine cells in the gastrointestinal tract of the barking deer, Muntiacus muntjak. Anatomia, Histologia, Embryologia 40, 365-374.

Ahlman, H., Nilsson, O., 2001. The gut as the largest endocrine organ in the body. Annals of Oncology 12, S63-S68.

Aksoy, A., Cinar, K., 2009. Distribution and ontogeny of gastrin-and serotonin-immunoreactive cells in the proventriculus of developing chick, Gallus gallus domestica. Journal of Veterinary Science 10, 9-13.

Althnaian, T.A., Ali, A.M., Bahr, S.M.E., 2019. Pancreatic hormones of dromedary camel: immunohistochemical localisation. Journal of Camel Practice and Research 26, 15-20.

Asar, M., Bayram, Z., Korgun, E.T., Tertemiz, F., Akkoyunlu, G., Demir, R., 2004. Immunocytochemical detection of synaptophysin in enteric neurones during prenatal development in the rat stomach. Anatomia, Histologia, Embryologia 33, 135-140.

Bargooth, A.F., Ali-Jebori, J.G.A., Al-Badri, A.M., Al-Yasari, A.M.R., Zegyer, E.A., 2020. Immunohistochemical study to detect glucagon and insulin hormones in pancreas of camel and buffalo. Veterinary World 13, 354-359.

Bertelli, E., Bendayan, M., 2005. Association between endocrine pancreas and ductal system. More than an epiphenomenon of endocrine differentiation and development? Journal of Histochemistry and Cytochemistry 53, 1071-1086.

Buffa, R., Rindi, G., Sessa, F., Gini, A., Capella, C., Jahn, R., Navone, F., De Camilli, P., Solcia, E., 1987. Synaptophysin immunoreactivity and small clear vesicles in neuroendocrine cells and related tumours. Molecular and Cellular Probes 1, 367-381.

Crowe, A. R., Yue, W., 2019. Semi-quantitative Determination of Protein Expression using Immunohistochemistry Staining and Analysis: An Integrated Protocol. Bio-protocol 9, e3465.

Dirice, E., De Jesus, D.F., Kahraman, S., Basile, G., Ng, R.W.S., El Ouaamari, A., Teo, A.K.K., Bhatt, S.,Hu.,J., Kulkarni, R.N., 2019. Human duct cells contribute to β cell compensation in insulin resistance. JCI Insight 4, e99576.

El-Salhy, M., Mazzawi, T., Hausken, T., Hatlebakk, J.G., 2016. Interaction between diet and gastrointestinal endocrine cells. Biomedical Reports 4, 651-656.

García, A., Masot, J., Franco, A., Gázquez, A., Redondo, E., 2012. Histomorphometric and immunohistochemical study of the goat rumen during prenatal development. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology 295, 776-785.

García, A., Masot, J., Franco, A., Gázquez, A., Redondo, E., 2014. Immunohistochemical evaluation of the goat forestomach during prenatal development. Journal of Veterinary Science 15, 35-43.

Hafez, S. A., Zaghloul, D., Caceci, T., 2015. Immunohistochemical identification of the endocrine cells in the pancreatic islets of the camel, horse and cattle. Eur. J. Anat. 19, 27-35.

Kyriakopoulos, G., Mavroeidi, V., Chatzellis, E., Kaltsas, G.A., Alexandraki, K.I., 2018. Histopathological, immunohistochemical, genetic and molecular markers of neuroendocrine neoplasms. Annals of Translational Medicine, 6, 1-13.

Masaad, G.A.M. 2007. A comparative morphological study on the pancreas of the dromedary camel (*Camelus dromedarius*) and the donkey (Equus asinus). Thesis, Master of Veterinary Medicine, University of Khartoum, Khartoum, Sudan.

Merkwitz, C., Blaschuk, O.W., Schulz, A., Lochhead, P., Meister, J., Ehrlich, A., Ricken, A.M., 2013. The ductal origin of structural and functional heterogeneity between pancreatic islets. Progress in Histochemistry and Cytochemistry 48, 103-140.

Mohan, H., Gasner, M., Ramesh, N., Unniappan, S., 2016. Ghrelin, ghrelin-O-acyl transferase, nucleobindin-2/nesfatin-1 and prohormone convertases in the pancreatic islets of Sprague Dawley rats during development. Journal of Molecular Histology 47, 325-336.

Nafikov, R.A., Beitz, D.C., 2007. Carbohydrate and lipid metabolism in farm animals. The Journal of nutrition 137, 702-705.

Park, I.-S., Bendayan, M., 1994. Endocrine cells in the rat pancreatic and bile duct system: alteration in diabetes. Pancreas 9, 566-573.

Portela-Gomes, G.M., Stridsberg, M., Johansson, H., Grimelius, L., 1999. Co-localization of synaptophysin with different neuroendocrine hormones in the human gastrointestinal tract. Histochemistry and Cell Biology 111, 49-54.

Pyarokhil, A.H., Sasaki, M., Tomikawa, S., Maetani, A., Yuhara, K., Kobayashi, Y., Kitamura, N., 2017. Immunohistochemical study of endocrine cells in the stomach and small intestine of the lion (Panthera leo). Mammal Study 42, 179-184.

Raji, A.R., 2011. Morphological and histochemical investigation of the camel (*Camelus dromedarius*) abomasal mucous membrane by light and scanning electron microscopy (SEM). Iranian Journal of Veterinary Research 12, 304-308.

Redecker, P., Jörns, A., Jahn, R., Grube, D., 1991. Synaptophysin immunoreactivity in the mammalian endocrine pancreas. Cell and Tissue Research 264, 461-467.

Rindi, G., Leiter, A.B., Kopin, A.S., Bordi, C., Solcia, E., 2004. The "normal" endocrine cell of the gut: changing concepts and new evidences. Annals of the New York Academy of Sciences 1014,

1-12.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Method, 9, 671-675.
Seiron, P., Wiberg, A., Kuric, E., Krogvold, L., Jahnsen, F.L., Dahl-Jørgensen, K., Skog, O., Korsgren, O., 2019. Characterisation of the endocrine pancreas in type 1 diabetes: islet size is maintained but islet number is markedly reduced. The Journal of Pathology: Clinical Research 5, 248-255.
Solcia, E., Rindi, G., Buffa, R., Fiocca, R., Capella, C., 2000. Gastric endocrine cells: types, function and growth. Regulatory Peptides 93, 31-35.
Stengel, A., Hofmann, T., Goebel-Stengel, M., Lembke, V., Ahnis, A., Elbelt, U., Lambrecht, N.W.G., Ordemann, J., Klapp, B.F., Kobelt, P., 2013. Ghrelin and NUCB2/nesfatin-1 are expressed in the same gastric cell and differentially correlated with body mass index in obese subjects. Histochemistry and Cell Biology 139, 909-918.
Trandaburu, T., Trandaburu, I., 2009. The immunohistochemical localization of synaptophysin protein (p38) in the gastro-entero-pancreatic (GEP) system of reptiles. Acta Histochemica 111, 476-487.
Türk, S., Çınar, K., Öztop, M., 2019. Distribution and relative frequency of immunohistochemically detected endocrine cells in the stomach of New Zealand White rabbit (*Oryctolagus cuniculus*). Iranian Journal of Veterinary Research 20, 39-45.
Valtorta, F., Pennuto, M., Bonanomi, D., Benfenati, F., 2004. Synaptophysin: leading actor or walk-on role in synaptic vesicle exocytosis? Bioessays 26, 445-453.
Van de Casteele, M., Leuckx, G., Baeyens, L., Cai, Y., Yuchi, Y., Coppens, V., De Groef, S., Eriksson, M., Svensson, C., Ahlgren, U., 2013. Neurogenin 3+ cells contribute to β-cell neogenesis and proliferation in injured adult mouse pancreas. Cell Death and Disease 4, e523-e523.
Waldum, H. L., Ringnes, E., Nordbø, H., Sørdal, Ø., Nordrum, I. S., Hauso, Ø., 2014. The normal neuroendocrine cells of the upper gastrointestinal tract lack E-cadherin. Scandinavian journal of gastroenterology 49, 974-978.
Wieczorek, G., Pospischil, A., Perentes, E., 1998. A comparative immunohistochemical study of pancreatic islets inlaboratory animals (rats, dogs, minipigs, nonhuman primates). Experimental and Toxicologic Pathology 50, 151-172.
Wiedenmann, B., Franke, W.W., Kuhn, C., Moll, R., Gould, V.E., 1986. Synaptophysin: a marker protein for neuroendocrine cells and neoplasms. Proceedings of the National Academy of Sciences 83, 3500-3504.
Wiedenmann, B., Waldherr, R., Buhr, H., Hille, A., Rosa, P., Huttner, W. B., 1988. Identification of gastroenteropancreatic neuroendocrine cells in normal and neoplastic human tissue with antibodies against synaptophysin, chromogranin A, secretogranin I (chromogranin B), and secretogranin II. Gastroenterology 95, 1364-1374.
Zhao, C.-M., Furnes, M. W., Stenström, B., Kulseng, B., Chen, D., 2008. Characterization of obestatin-and ghrelin-producing cells in the gastrointestinal tract and pancreas of rats: an immunohistochemical and electron-microscopic study. Cell and Tissue Research 331, 575-587.