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RESEARCH ARTICLE

Distribution of Enteroendocrine Cells in the Small Intestine of the One Humped Camel (*Camelus dromedarius*)

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Abstract

Enteroendocrine cells in small intestine plays as endocrine portion by secreting some hormones that works key roles in the regulation of certain important organs. The aim of this study was to examine in detail about distribution of Enteroendocrine cells in the small intestine of the camel. The regional distribution of endocrine cells in the small intestine of the camel, *Camelus dromedarius*, were investigated using immunohistochemical techniques. Specimens from ten dromedarian camels (*Camelus dromedarius*) of both sexes with age ranging from 2-8 years were investigated. The immunohistochemistry was performed using Chromogranin A (ChA), and four types of hormones. Immunodetection methods demonstrated that in the camel small intestine, glucose-dependent insulinotropic peptide (GIP), Glucagon-like peptide-1 (GLP-1), Glucagon-like peptide-2 (GLP-2) and Cholecystokinin (CCK) were expressed in a subset of epithelial cells along the crypt villus axis. The cells that contained gut hormones appeared to be either triangular or flask-like in shape suggesting that they are enteroendocrine cells. Immunohistochemistry of serial sections showed that ChA, which is a specific marker for enteroendocrine cells, was indeed expressed with GIP, GLP-1, GLP-2 and CCK-8 confirms the site of expression to be in enteroendocrine cells. There is an aboral decrease in K-cells and I-cells along of the camel small intestine but an increase in L-cells. The regional distribution of immunoreactive cells in the one humped camel is essentially similar to those of other mammals. In conclusion, the study results showed that the small intestine of camel has distinctive characters immunodetection. Therefore, further experimental and physiological studies are needed.

Introduction

In the tropical area (Africa and Asia), the one humped camel (*Camelus dromedaries*) is mostly present in Arabian countries and known as the ship of the desert (Azwai *et al.,* 1996). The camel is ruminant, but its stomach differs morphologically from that of other ruminants (Eerdunchaolu *et al.,* 2001). Gut hormones have a significant effect on the digestive functions of the intestine (Fujita and Kobayashi, 1977). Many immunohistochemistry studies have carried on the distribution of endocrine cells in the intestinal tract of human, cattle, pigs, lesser mouse deer, sheep, horse, water buffalo, babirusa and camel (Cristina *et al.,* 1978; Sjolund *et al*., 1983; Calingasan *et al.,* 1984; Kitamura *et al.,* 1984; Ito *et al.,* 1987; Agungyriyono *et al.,* 2000; Eerdunchaolu *et al.,* 2001; Ham, 2002; Ali *et al.,* 2007; Daly *et al*., 2012). Studies on the enteroendocrine cells of the small intestine of the one humped camel (*Camelus dromedaries*) are virtually lacking. The present paper were undertaken to study the regional distribution of enteroendocrine cells in the small intestine of the camel using immunohistochemistry techniques.

Materials and methods

Samples were obtained from small intestine of ten dromedarian camels (*Camelus dromedarius*; five females and five males, aged 2-8 years; 250-400kg). Tissue samples were collected from small intestine approximately 50 cm in length were removed from the proximal (distal to the pylorus), mid (half way along the small intestine), and distal (proximal to the ileocaecal junction) intestine. Sections were opened longitudinally, rinsed in ice-cold 0.9% (w/v) NaCl pH 7.4, and blotted with paper towels to remove excess mucous. Samples of the tissue were fixed in 10% formaldehyde, dehydrated through an ethanol-xylene serirs and embedded in paraffin for histological examinations and immunohistochemical studies. Sections were cut at 5mm in thickness. Slides, containing wax embedded camel small intestinal tissue, were dewaxed in 100% xylene for 3 x 10 minutes each. The tissue was placed twice in 100% ethanol for 2 x 10 minutes. Sections were removed, allowed to air dry for 10 minutes and were circled with ImmEdge Hydrophobic Pen and allowed to dry for 10 minutes. Subsequently, they were placed 2 x 5 minutes in 70% ethanol. Slides were then rehydrated twice in distilled H2O for 5 minutes each. Slides were immersed in antigen retrieval buffer (10 mM Tris/HCl pH 10.0) and autoclaved (Series A1200086, LMS CONS. Ltd, Germany) 2 x 15 minutes at 121°C and 15 psi. Subsequently, slides were allowed to cool in antigen retrieval buffer for 30-60 minutes at room temperature and washed for 3 x 5 minutes in phosphate buffer saline (PBS). Non specific antibody binding sites were blocked by incubating the tissue sections for 1 hour in the blocking solution 10% (v/v) donkey serum in a humidified chamber at room temperature. Sections were incubated overnight at 4°C with primary antibodies (Table 1). For double-immunostaining, primary antibodies raised in different species were mixed with one another without changing the final required concentration and were incubated at 4○C overnight. Each slide was then washed in PBS for 5 x 5 minutes. FITC-conjugated IgG/IgY and Cy3-conjugated IgG/IgY (Table 1) (Stratech, Scientific Limited, Suffolk, UK) were used at a dilution of 1:500 for 1 hour incubation at room temperature. Finally, slides were washed with PBS for 5 x 5 minutes and mounted in Vectashield Hard Set Mounting Media with DAPI (Vector Laboratories Ltd, Peterborough, UK). Sections were visualised using an epifluorescence microscope (MEIJI TECHNO, Model MT4300, Japan) and images were captured with a Canon digital camera (DS126371, Canon INC, Japan). Images from serial sections were merged using Imaging Products Laboratory imaging software (BioVision Technologies, Exton, PA, USA). Omission of primary antibody was routinely used as a control.

Table1. Primary and secondary antibodies were used.

Primary antibody	Host	Dilution	Clonality and Source
Anti-chromogranin A	Rabbit	1:100	Polyclonal, chromogranin A (H-300): sc-13090 Santa Cruz Biotechnology, INC., CA, USA
Anti-chromogranin A	Goat	1:100	Polyclonal, chromogranin A (C-20): sc-1488, Santa Cruz Biotechnology, INC., CA, USA.
Anti-GIP	Goat	1:100	Polyclonal, GIP (Y-20): sc-23554, Santa Cruz Biotechnology, INC., Santa Cruz, CA, USA.
Anti-GLP-1	Goat	1:100	Polyclonal, GLP-1 (C-17): sc-7782, Santa Cruz Biotechnology, INC., Santa Cruz,CA, USA
Anti-GLP-2	Goat	1:100	Polyclonal, GLP-2 (C-20): sc-7781, Santa Cruz Biotechnology, INC., Santa Cruz,CA, USA
Anti-CCK-8	Rabbit	1:200	Polyclonal, CCK (C2581): Sigma, Saint Louis, Missouri, USA.

Secondary antibody	Label	Dilution	Source
Donkey anti-rabbit IgG	Cy3	1:500	Cyanine-conjugated IgG (711-165-152), Stratech Scientific Limited, Suffolk, UK.
Donkey anti-rabbit IgG	FITC	1:500	Fluorescein-conjugated IgG (711-095-152), Stratech Scientific Limited, Suffolk, UK.
Donkey anti-goat IgG	Cy3	1:500	Cyanine-conjugated IgG (705-166-147), Stratech Scientific Limited, Suffolk, UK.
Donkey anti-goat IgG	FITC	1:500	Fluorescein-conjugated IgG (705-095-147), Stratech Scientific Limited, Suffolk, UK.

Results

Expression of GIP, GLP-1, GLP-2 and CCK in the intestine of camels

The immunohistochemistry was carried on the different part of dromedarian camel small intestine (proximal, mid and distal) using 4 types of hormones which were GIP, GLP-1, GLP-2 and CCK-8 (Table 1). Wax embedded sections of intestine from camel were labelled with specific antibodies against to GIP, GLP-1, GLP-2 and CCK as described earlier in Material and Methods. Immunohistochemistry showed clearly that the gut hormones were expressed solely in a subpopulation of cells along the villus in the proximal, mid and distal parts of small intestine of the one-humped camel (Fig. 4.1). There is an aboral decrease in K-cells containing GIP and I-cells containing CCK along of the camel intestine, but an increase in L-cells containing GLP-1 and GLP-2.

The enteroendocrine cells that expressed gut hormones were the open type which the apex of the cells presents brush border membrane (microvilli) and found in the epithelial lining of the lumen. Gut hormones labelling were specific; it was not observed when the primary antibodies for GIP, GLP-1, GLP-2 and CCK were omitted (Fig. 4.1).

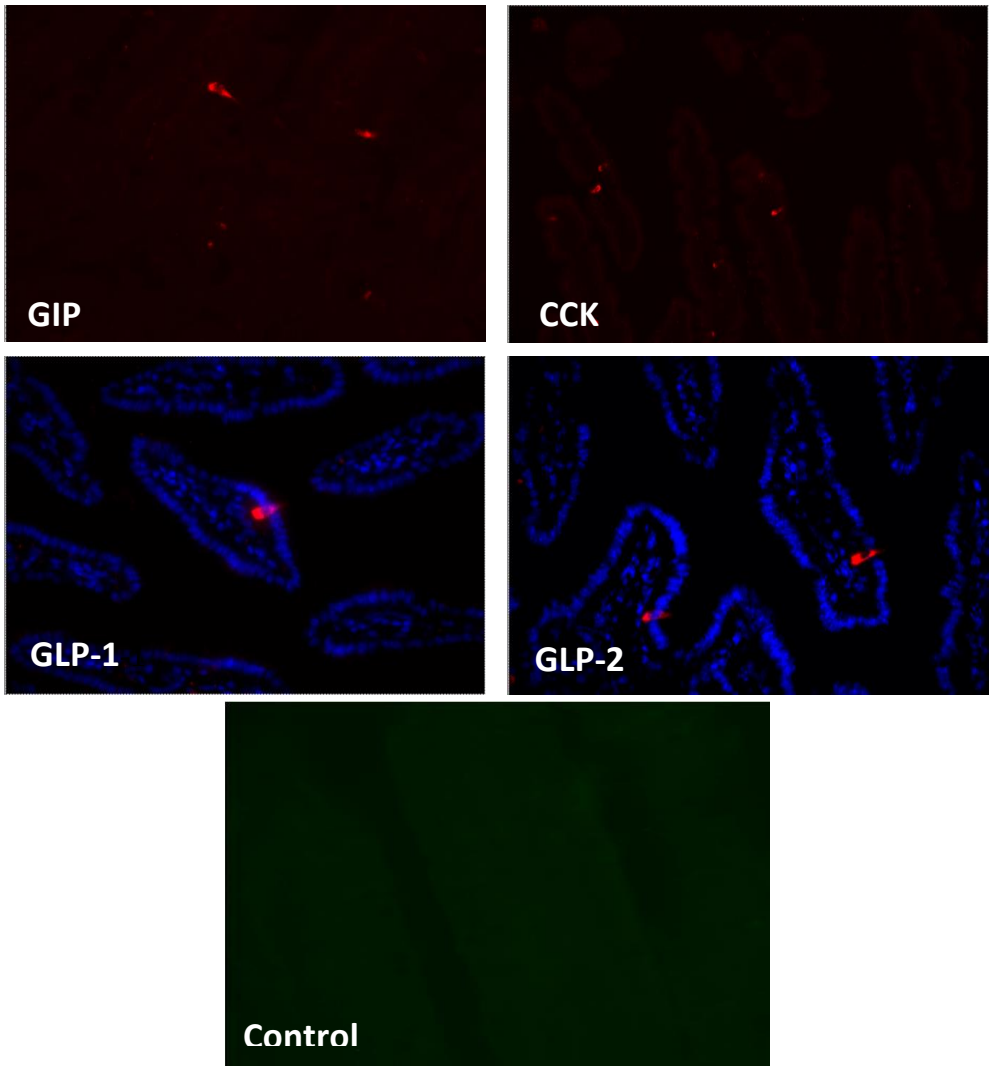

Figure 4.1: **Wax embedded sections of small intestine were probed with the antibodies to GIP, GLP-1, GLP-2 and CCK. Typical images showing gut hormones red in a subset of camel intestinal cells. Control image show that there is no labelling for gut hormones. Images are 200 X magnified. Nuclei are stained blue with 4', 6-diamidino-2-phenylindole (DAPI).**

Co-localisation of gut hormones with Chromogranin A

The presence of the gut hormones have demonstrated in small intestine of camels. The cells that contain gut hormones appear to be either triangular or flask-like in shape and located mainly in villi. The shape of these cells suggested that they may be enteroendocrine cells. Consequently, to investigate the cell type expressing gut hormones, double immunohistochemistry was employed with an antibody to chromogranin A (ChA), a classical marker for endocrine cells. With double immunostaining technique, primary antibodies to each protein were raised in different species. This allows labelling with two secondary antibodies anti-rabbit IgG labelling with one fluorochrome and anti-goat IgG labelled with another. When viewed singly, staining was red for one protein and green for another. When the images were merged, areas of co-expression showed as yellow/ orange.

1- Co-localisation of GIP and ChA in camel small intestine

Wax embedded small intestine sections from camel were incubated with antibodies to GIP and ChA. Immunostaining showed that enteroendocrine K-cells containing GIP and ChA were co-expressed in the same cells in camel small intestine (Fig. 4.2)

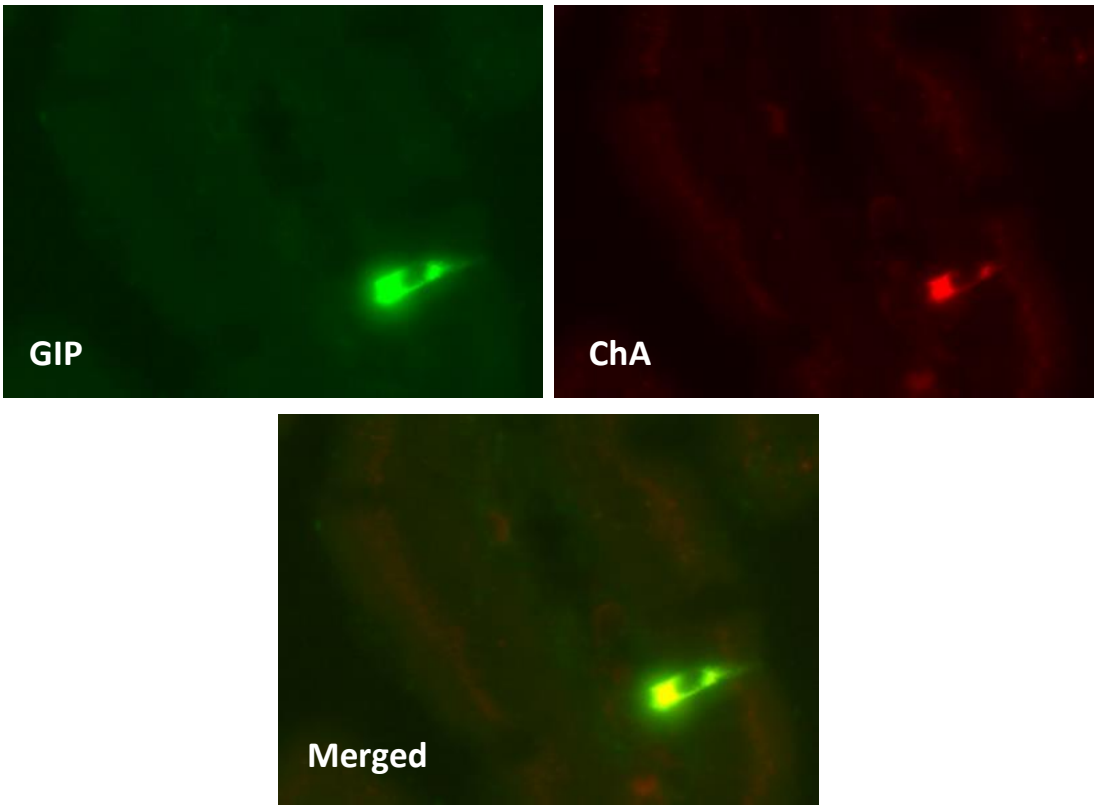

Figure 4.2: Wax sections of camel small intestine were labelled with the primary antibodies to GIP and ChA. A representative immunofluorescence images showing co-localisation of GIP (green) with ChA (red). When the sections were overlaid, GIP and ChA were shown to be co-expressed in a same cell (yellow). Images are 400 X magnified.

2- Co-expression of GLP-1/GLP-2 and ChA in camel small intestine

Wax embedded sections were probed with antibodies to GLP-1 or GLP-2 and ChA. Immunohistochemistry results showed that enteroendocrine L-cells containing GLP-1/ GLP-2 resided with ChA in the same cells in camel intestine (Fig. 4.3).

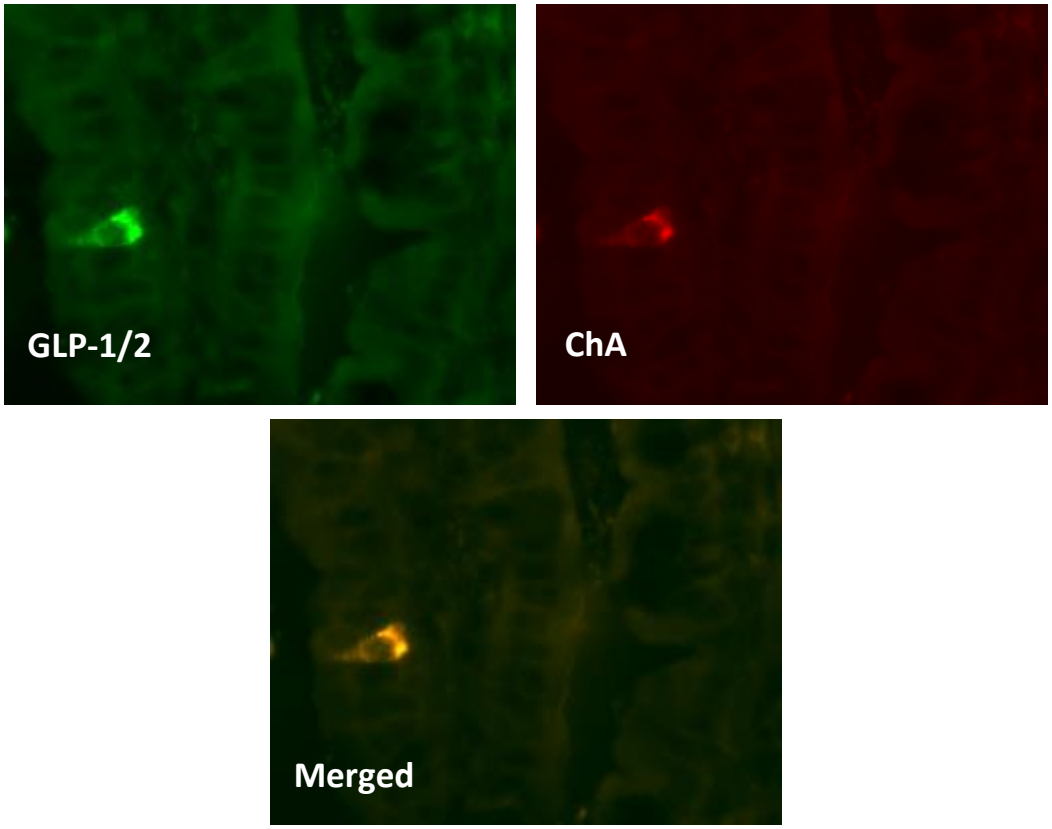

Figure 4.3: Typical immunofluorescence images show enteroendocrine L-cells containing GLP-1/2 (green) and ChA-positive cells (red). The merged GLP-1/2 and ChA showed that the gut hormones and ChA were localised in the same cells (yellow) staining. Images are 400 X magnified.

3- CCK coexpressed with ChA in camel small intestine

Wax embedded sections of small intestine from camel were labelled with antibodies to CCK and ChA (Fig. 4.4). Double immunostaining showed that enteroendocrine I-cells containing CCK -positive cells are co-localised with ChA in camel intestine (Fig. 4.4).

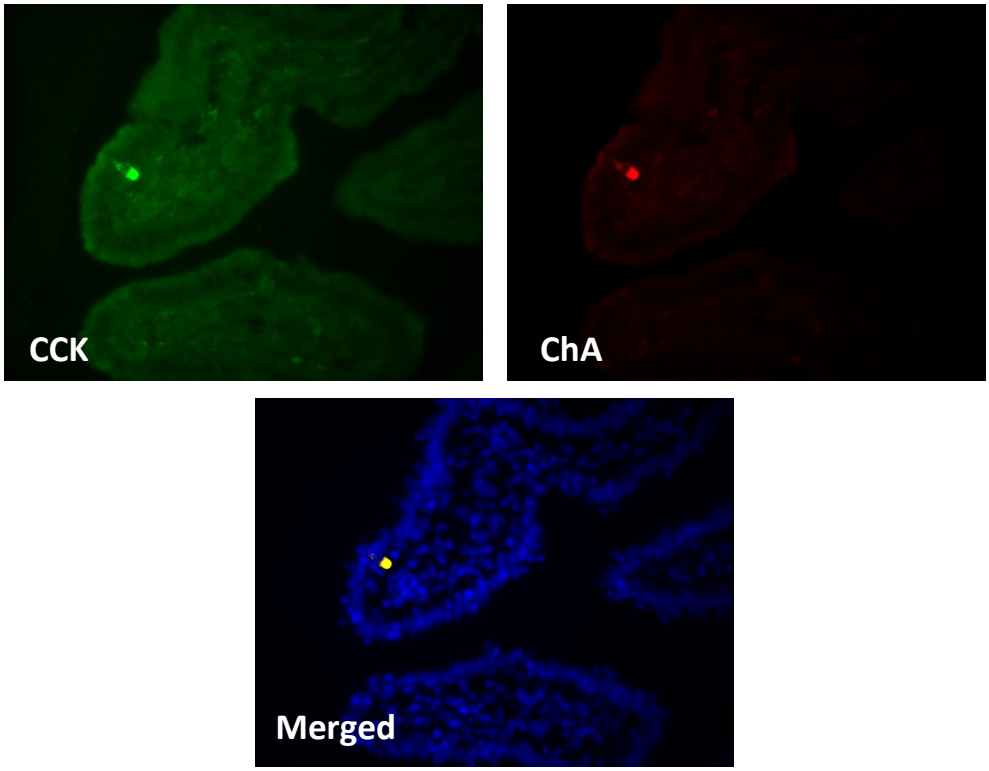

Figure 4.4: Colocalization of CCK and ChA in a subset of epithelial cells of the intestinal mucosa of camels. Double immune-staining of wax sections of small intestine was used for immunofluorescent detection of CCK (green) and ChA (red). The images have been merged and show colocalization of CCK and ChA in the same cells (yellow). C: nuclei are stained blue with 4', 6-diamidino-2-phenylindole (DAPI).

Discussion

Enteroendocrine cells secrete hormones and peptides and the effect of these hormones and peptides on food intake and appetite, the regulation of glucose homeostasis, gut motility and various other physiological functions (Mellitzer and Gradwohl 2011). In the small intestine, neuroenteroendocrine cells are highly specialised mucosal cells that produce a wide range of hormones with specific regional distribution and play a vital role in the function of the digestive system with enteric nervous system (Ali *et al.*, 2007). The endocrine cells in each part of the gastrointestinal tract differ remarkably between animal species in term of regional distribution, relative frequency and cell type (Ham, 2002). Enteroendocrine cells constitute 1% of the cells lining the intestinal epithelium, and there are twenty or more subtypes of enteroendocrine cells based on the major products they secrete (Moran *et al.*, 2010). K-enteroendocrine cells secrete GIP; I-enteroendocrine cells secrete CCK-8, while L-enteroendocrine cells secrete GLP-1and GLP-2 in response to dietary carbohydrates, amino acids and lipids (Jang *et al.*, 2007; Daly *et al.*, 2012 and 2013). In the present study, we have demonstrated the expression and distribution of four types of neuroendocrine cells in the proximal, mid and distal parts of small intestine of the one-humped camel using immunohistochemical techniques. This study, however, is the first to clarify immunohistochemically the type, and distribution of neuroenteroendocrine cells in the small intestine of the one humped camel.

In the present study, we have demonstrated that GIP was expressed solely in a subset of cells along the villus in the intestine of camels (Fig 4.1). The cells expressing GIP appeared to be either triangular or flask-like shape. Moreover, we have demonstrated that cells possessing the GIP also coexpressed with chromogranin A (Fig 4.2). Co-expression of the GIP with chromogranin A, a classical marker of enteroendocrine cells confirms the site of expression to be in enteroendocrine cells. This pattern of distribution of the GIP has also been shown in other mammals including human (Theodorakis *et al*., 2006), buffalo (Lucini *et al*., 1999), rabbit (Keast *et al*., 1987), sheep and cow (Calingasan *et al*., 1984; Kitamura *et al*., 1984), pig (Moran *et al.*, 2010), dog (Damholt *et al*., 1999) and horse (Daly *et al.*, 2012). In the mid and distal small intestine, the K cell was an aboral decrease along the length of the camel intestine. This pattern of distribution of the GIP in the mid and distal small intestine has been documented in other mammals by (Moran *et al*., 2010).

The data indicate that GLP-1 and GLP-2 are expressed in a subpopulation of cells along the villus in the small intestine of camels (Fig 4.1). Similar findings concluded that GLP-1 and GLP-2 have been demonstrated on dromedary camel (Ali *et al*., 2007), pig, calf and sheep (Moran *et al*., 2010 and 2014), horse (Daly *et al*., 2012). Furthermore, we have demonstrated that cells possessing the GLP-1/2 also colocalised with chromogranin A (Fig 4.3). In the proximal, mid and distal small intestine of camels, the enteroendocrine L-cell was an aboral increase along the length of the camel intestine. This pattern of distribution of the GLP-1/2 in small intestine has been seen in other mammals by (Moran *et al*., 2010). In the duodenum of camel, the presence of the GLP-1/2 was beneficial for the metabolism of the carbohydrate. The study results was agreed with those of Agungpriyono *et al*., (2000) who reported low level of expression in gastrointestinal of babirusa and low expression of GLP-1/2 in duodenum of the two humped camel (Eerdunchaolu *et al*., 2001).

Enteroendocrine I-cells released a peptide hormone called Cholecystokinin (CCK) from the duodenum (Field *et al*., 2010 and Rindi *et al*., 2004). Our results have shown that in the proximal small intestine, CCK was localized in a subset of cells along the villus in the intestine of camels (Fig 4.1). Most of the CCK-immunoreactive (IR) cells were flask shaped with apices pointing towards the lumen of the gut (Fig 4.1). This pattern of expression of the CCK has been shown in other mammals (Ali *et al.*, 2007 and Daly *et al.*, 2013). Furthermore, we have confirmed the site of expression to be in enteroendocrine cell by doing double immunostaining and the results showed that cells possessing the CCK also coexpressed with chromogranin A (Fig 4.4). In the mid small intestine of camels, the enteroendocrine I-cell that expressed the CCK was decreased. This finding was supported by Field *et al*., (2010), Ali *et al*., (2007) and Rindi *et al*., (2004) who reported that CCK expressed and released from enteroendocrine I-cells of the jejunum. In the distal part of small intestine of camels, CCK-IR cells were rarely detected. A similar pattern of expression for I-enteroendocrine cells that expressed CCK were observed in all region of small and large intestine of two frugivorous (Artibeus cinerius and Sturnira lilium) and (*Camelus bactrianus*) (Dos Santos *et al*., 2008 and Eerdunchaolu *et al*. 2001).

Our results support the important digestive role of endocrine cells in the gut of camel. In this study, however, three types of endocrine cells that secrete, at least, GIP, GLP-1, GLP-2 and CCK-8 were identified in the small intestine. The localised, relatively stable presence of GIP, GLP1/2 and CCK in the upper small intestine may be related to the role of these hormones in the stimulation of intestinal and gallbladder smooth muscle and pancreatic secretion.

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