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Distribution of neuroendocrine cells in the small and large intestines of the one-humped camel (*Camelus dromedarius*)

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Abstract

The distribution and relative frequency of neuroendocrine cells in the small and large intestines of one-humped camel were studied using antisera against 5-hydroxytryptamine (5-HT), cholecystokinin (CCK-8), somatostatin (SOM), peptide tyrosine tyrosine (PYY), gastric inhibitory polypeptide (GIP), neuronal nitric oxide synthase (nNOS), gastrin releasing peptide (GRP), substance P (SP), and neurokinin A (NKA). Among these cell types, CCK-8 immunoreactive (IR) cells were uniformly distributed in the mucosa, while others showed varied distribution in the villi or crypts of the small intestine. Immunoreactive cells like 5HT, CCK-8, and SOM showed peak density in the villi and crypts of the small intestine and in the colonic glands of the large intestine, while cells containing SP were discerned predominately in the crypts. 5-HT, CCK-8 and SOM cells were mainly flask-shaped and of the open-variety, while PYY and SP immunoreactive cells were mainly rounded or basket-shaped and of the closed variety. Basically the distribution pattern of the endocrine cells in the duodenum, jejunum and colon of the one-humped camel is similar to that of other mammals. Finally, the distribution of these bioactive agents may give clues as to how these agents aid in the function of the intestinal tract of this desert animal.

Keywords: Camel; Duodenum; Jejunum; Colon; Neuroendocrine cells; Immunohistochemistry

1. Introduction

The one-humped camel is a typical desert animal that has developed sophisticated physiological adaptation for coping with heat, feed and water scarcity in its dry and rough habitat. These adaptations seem to depend on its ability to tolerate severe dehydration and to economize the meagre water availability (Macfarlane et al., 1963). The mechanism used is not well understood, but probably involves several organ systems including the gastrointestinal system, which is well known for fluid and electrolyte transport. The neuroendocrine cells, dispersed among the epithelial cells of the gastrointestinal tract, together with the enteric nervous system play a vital role in the function of the digestive system. Great interest in the role of these endocrine cells and the enteric nervous system increased following the demonstration of their involvement as neurotransmitters in the regulation of muscular movement, secretion of intestinal glands and control of vascular permeability of the digestive system (Ham, 2002). The fluids and electrolytes that are released into the mammalian gut are enhanced

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mainly by acetylcholine, vasoactive intestinal polypeptide (Mailman, 1978; Brunsson et al., 1995) and substance P (Greenwood et al., 1990) and to some extent by calcitonin gene related peptide, cholecystokinin-8, and galanin (Polak and Bloom, 1986). Noradrenaline, neuropeptide Y (MacFadyen et al., 1986), peptide YY (Lundberg et al., 1982; Playford and Cox, 1996) and somatostatin (Guandalini et al., 1980; Dharmsathaphorn et al., 1980) enhance absorption and reduce secretion of water and electrolyte from the small gut. Immunohistochemical studies have been performed to investigate the distribution and relative frequency of neurons and neuroendocrine cells containing these neurotransmitters and neuropeptides in the gastrointestinal tract of several mammals including humans (Cristina et al., 1978), horse (Kitamura et al., 1984), pigs (Ito et al., 1987), lesser mouse deer (Agungpriyono et al., 1994) and rat (Adeghate et al., 1995) in order to elucidate their function. In view of the fact that neurotransmitters and neuropeptides such as serotonin, acetylcholine, somatostatin, PYY and substance P have been implicated in the regulation of fluid across the intestinal epithelium, it was tempting to examine the pattern of distribution of these neurotransmitters and neuropeptides in the small and large intestines of the one-humped camel, an animal known to be able to strive in the desert. Therefore, the aim of this study was to determine the distribution profile of neuroendocrine cells in the duodenum, jejunum, and colon of the one-humped camel. The distribution of these cells might be related to the regulatory characteristics of the digestive tract and provides insights into the roles these organs may play in water conservation of this desert animal.

2. Materials and methods

2.1. Animals

Five healthy male dromedarian camels, aged 4–6 years and weighing 350–450 kg were used for this study. They were slaughtered for food at the local abattoir. Pieces of the small and large intestines were removed and transferred expeditiously into Zamboni's fixative (Zamboni and De Martino, 1967).

2.2. Tissue preparation

Segments of small and large intestines collected and fixed in zamboni solution were kept in the fixative overnight at 4 °C. After 24 h, transverse sections, each measuring 1.0 cm in length were cut and embedded in paraffin according to established method (Adeghate et al., 2003). The duodenal and jejunal portions of the small intestines have been used for this study because like in most mammalian species, they account for 95–98% of the camel small gut (Smuts and Bezuidenhout, 1987). Random portions of the proximal colon were used for this study, because there are no reported differences between different parts of the large bowel of the one-humped camel as compared to other mammals in which gross anatomical differences were observed (Smuts and Bezuidenhout, 1987).

2.3. Immunohistochemistry

Serial sections of 7.0 μm thicknesses were cut from blocks of the duodenum, jejunum, and colon with a Shandon A325 rotatory microtome. Sections were stained immunohistochemically using the avidin–biotin-peroxidase Complex method (Hsu et al., 1981; Adeghate et al., 2001). Briefly, endogenous peroxidase activity was blocked by incubating the sections for 30 min in 3.3% hydrogen peroxide in absolute methanol. The sections were incubated with the specific antisera (Table 1) all of which were raised in rabbit and then washed in 0.1 M PBS 3 times for 5 min (3×5 min) before incubation for 1 h in prediluted biotinylated anti rabbit, secondary antibody (Shandon, Pittsburgh, USA). Sections were again washed 3×5 min in PBS, followed by incubation in prediluted streptavidin peroxidase reagent (Shandon, Pittsburgh, USA) for 1 h. The sections were subsequently washed in two changes of 0.1 M PBS and a third wash in 0.1 M phosphate-buffer (PB). Peroxidase activity was demonstrated with DAB (1.0 ml of diaminobenzidene hydrochloride (Sigma, St. Louis, USA) to which 7.5 μl of 30% H_2O_2 with 1 ml of 3.5% nickel chloride had been added and diluted to 50 ml with PB), for 3–5 min in a hooded incubator. Sections were finally washed in 0.1 M PB, air-dried and dehydrated in ethanol up to 100%, cleared in xylene and coverslipped using Cytoseal 60 mounting medium (Stephens Scientific, Riversdale, NJ, USA). The slides were examined on a Zeiss Axiophot photomicroscope.

Table 1
Types and sources of antisera used

Antiserum	Type	Dilution	Source
5-HT	Synthetic/ AES 308	1:1500	Harlan Sera Lab, UK
CCK-8	Synthetic	1:2000	Peninsula Labs, USA
SOM	Synthetic/ AES 313	1:1000	Harlan Sera Lab, UK
GIP	Synthetic	1:1500	Guildhay Antisera Ltd, UK
GRP	Synthetic	1:1500	Guildhay Antisera Ltd, UK
NKA	Synthetic	1:2000	Affinity Res. Prod., UK
nNOS	Rabbit	1:1500	Chemicon Inc., CA, USA
PYY	Synthetic	1:2000	Peninsula Labs, USA
SP	Synthetic/ AES 320	1:1500	Peninsula Labs, USA

5-HT (5-hydroxytryptamine, serotonin); CCK-8 (cholecystokinin-8); SOM (somatostatin); GIP (gastric inhibitory polypeptide); GRP (gastrin releasing peptide); NAK (neurokinin A); nNOS (neuronal nitric oxide synthase); PYY (peptide tyrosine tyrosine); SP (substance P).