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AN IMMUNOHISTOCHEMICAL STUDY OF VARIOUS PEPTIDE-CONTAINING ENDOCRINE CELLS AND NEURONES AT THE EQUINE ILEOCAECAL JUNCTION

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ABSTRACT

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The ileocaecal junctions of 5 horses and 2 donkeys were examined by using antisera to the following peptides: somatostatin, glucagon, gastrin, neurotensin, vasoactive intestinal peptide (VIP), peptide histidine isoleucine (PHI), calcitonin gene-related peptide (CGRP), substance P (SP) and neuropeptide Y (NPY). Antisera to somatostatin, neurotensin and NPY demonstrated endocrine cells in the ileal- and caecal parts of the ileocaecal junction, while immunoreactivity for glucagon was demonstrated in endocrine cells of the ileal part only. Nerve cell bodies showing immunoreactivity to SP, VIP, CGRP and PHI were demonstrated in the myenteric and submucosal plexuses and were associated with small blood vessels in the submucosa of all the regions tested. Ramified nerve fibres in the submucosa immunoreactive to SP, VIP, CGRP and PHI extended to the mucosa and to small blood vessels in the submucosa. Nerve fibres showing immunoreactivity to SP, VIP and PHI extended to the circular smooth muscle layer of the ileocaecal junction.

INTRODUCTION

In the horse the ileocaecal junction contains a submucosal venous plexus (Schumer, 1953; Kotzé 1988b). It is postulated that upon engorgement the plexus acts as an additional closing mechanism.

The action of the autonomic nervous system on sphincters and blood vessels of the gastrointestinal tract is well established (Guyton, 1986). Currently bioactive peptides can be demonstrated in endocrine cells and neurones of the gut by means of immunohistochemical techniques. These peptides play a regulatory role in the overall function of the gastrointestinal tract (Furness & Costa, 1987). Kitamura, Yamada, Calingasan & Yamashita (1984), demonstrated various immunoreactive endocrine cells in the gastrointestinal tract of the horse, without any emphasis on the ileocaecal junction.

The purpose of this study was to establish the distribution of peptides in endocrine cells and neurones of the ileocaecal juntion of the horse, thereby furthering the understanding of the function of this area.

MATERIALS AND METHODS

Tissues were obtained from 5 thoroughbed horses, 7 months to 14 years of age, and 2 five year old donkeys of both sexes. The animals were clinically healthy, and were anaesthetized using 20 % chloral hydrate. Subsequently the left common carotid arteries were catheterized and the animals were sacrificed by exsanguination. The distal ileum, the ileocaecal junction and the surrounding caecum, together with the arteries supplying these areas were removed immediately after death. Specimens were fixed by perfusion using Bouin's fluid. From the ileocaecal junction 5 mm thick longitudinal strips were cut which included short portions of the ileum and caecum. The partially fixed specimens were transfered to fresh Bouin's fluid for 7 h at room temperature, dehydrated and imbedded in paraffin wax. Sections (6 µm) were cut and floated on glass slides pre-treated with poly-L-lycine (Van Noorden & Polak, 1983).

Sections were dewaxed, hydrated, endogenous peroxidase blocked by treating them with hydrogen peroxide in methanol (Burns, 1975), and transfered to Tris-saline buffer. Non-specific reaction sites were blocked by incubating the sections with non-immune serum for 10 min at room temperature. Peptides were identified by employing the peroxidase anti-peroxidase method (Sternberger, 1979). Details of specific antisera used in this study are listed in Table 1.

Positive tissues were employed as controls. The specificity of the primary antisera was validated by preabsorbing each antiserum with 20 µg of its parent antigen per 1 mℓ of diluted antiserum for 12 h at 4 °C.

Some of the immunostained sections were lightly counterstained with haematoxylin, whereas all the sections were dehydrated and mounted with DPX.

TABLE 1 Details of antisera used in this study

Antigen to which antiserum raised	Code No.	Working dilution	Source
Somatostatin	RPN1612	1:1 000	Amersham
Glucagon	RPN1602	1:1 000	Amersham
Gastrin	RPN1592	1:2 500	Amersham
Neurotensin	1000	1:1 000	J. M. Polak, London
Neuropeptide Y(NPY)	1263	1:500	J. M. Polak, London
Substance P (SP)	910	1:1 000	J. M. Polak, London
Vasoactive intestinal peptide (VIP)	925	1:1 000	J. M. Polak, London
Peptide histidine isoleusine (PHI)	1200	1:500	J. M. Polak, London
Calsitonin gene-related peptide (CGRP)	1209	1:500	J. M. Polak, London

RESULTS

Endocrine cells containing somatostatin, glucagon, gastrin, neurotensin and NPY respectively (Table 2) were identified in the ileocaecal junction. Conversely VIP, CGRP, SP and PHI were identified in neurones of the ileocaecal junction (Table 3).

Immunoreactive endocrine cells

Immunoreactive endocrine cells occurred in groups, and were unevenly distributed throughout the junctional area (Table 2). Results shown in Table 2 were recorded from areas where the highest concentrations of immunoreactive cells occurred.

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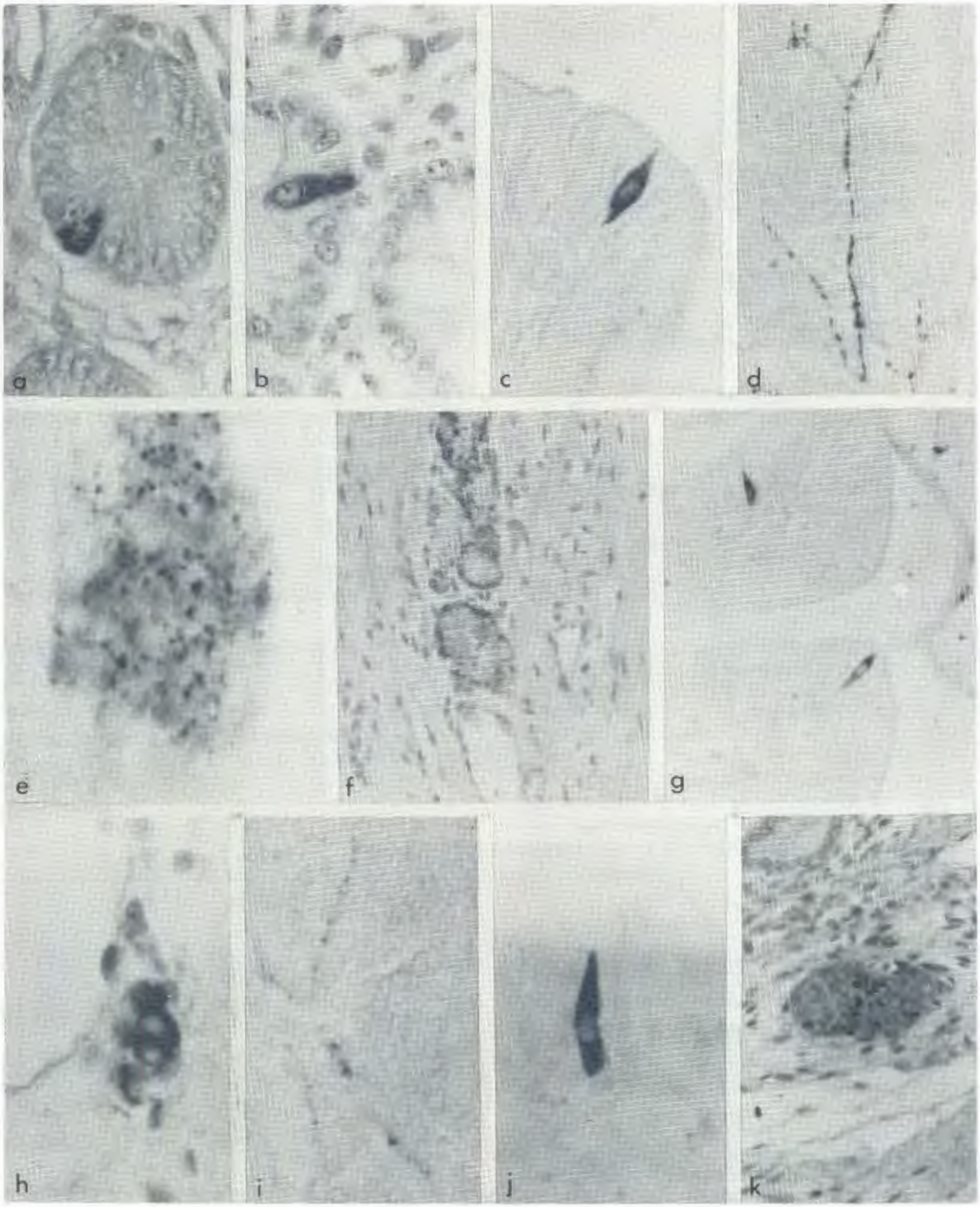

FIG. 1. Photomicrographs showing immunoreactive endocrine cells and neurones in the equine ileocaecal junction

a. A triangular-shaped endocrine cell demonstrating somatostatin immunoreactivity: × 600
b. A flask-shaped endocrine cell showing gastrin immunoreactivity: × 500
c. An elongated endocrine cell showing glucagon immunoreactivity: × 500
d. The beaded appearance of VIP immunoreactive nerve fibres extending into the mucosa: × 290
e. Nerve cell bodies showing VIP immunoreactivity in the submucosa: × 560
f. Nerve cell bodies in the submucosa showing SP immunoreactivity: × 290
g. Two elongated endocrine cells showing neurotensin immunoreactivity: × 300
h. Nerve cell bodies in the submucosa showing CGRP immunoreactivity: × 550
i. CGRP immunoreactive nerve fibres in the mucosa of the ileocaecal junction: × 260
j. An endocrine cell showing immunoreactivity to NPY: × 640
k. A small group of nerve cell bodies in the submucosa showing PHI immunoreactivity: × 290

Sparsely distributed somatostatin immunoreactive endocrine cells were demonstrated in both the ileal and caecal portions of the ileocaecal junctions (Table 2). The latter cells were mostly flask or triangular-shaped (Fig. 1a) and occurred in both the villi and crypts of the mucosa.

Gastrin immunoractivity was demonstrated in oval to flask-shaped endocrine cells of both the villi and crypts in the ileal and caecal parts of the junction (Fig. 1b). The concentration of the latter cells decreased towards the caecal part of the junction (Table 2).

In the villi and crypts of the ileal region of the junction, spindle or flask-shaped cells immunoreactive to glucagon were detected (Table 2; Fig. 1c).

Spindle or flask-shaped endocrine cells immunoreactive to neurotensin were present in the villi and crypts, with the largest concentration in the ileal part of the junction, gradually decreasing in number towards the caecal portion of the ileocaecal junction (Table 2; Fig. 1g).

Endocrine cells immunoreactive to NPY were demonstrated in villi and crypts of the ileal and caecal parts of the ileocaecal junction (Fig. 1j).

TABLE 2 The relative frequency and distribution of endocrine cells in the ileocaecal area of the horse and donkey

Antigen	Ileal part of junction	Caecal part of junction
Somatostatin	+	+
Glucagon	++	–
Gastrin	++	+
Neurotensin	++	+
NPY	++	+

\+ indicates 1–2 cells/mm²
+-
001 indicates 2–20 cells/mm²
– indicates absence of immunoreactivity

Immunoreactive neurones

Immunoreactivity to CGRP was observed in cell bodies of the myenteric and submucosal plexuses of the entire junctional area. In the submucosal plexus these cell bodies occurred as solitary cells or in small groups (Fig. 1h) and were in close proximity to the lamina propria and small blood vessels of the submucosa. Immunoreactive, ramified nerve fibres from these cells extended into the mucosa (Fig. 1i) and towards small blood vessels in the submucosa.

Cell bodies of nerve fibres immunoreactive for VIP were seen as groups (Fig. 1e) often close to submucosal blood vessels, or as solitary cells in the submucosal plexus. Ramified nerve fibres immunoreactive to VIP extended into the mucosa (Fig. 1d) whereas simple and ramified VIP immunoreactive nerve fibres reached the submucosal blood vessel walls. In the myenteric plexus small groups of VIP immunoreactive nerve cell bodies were detected as well as nerve fibres which extended to the circular smooth muscle layer of the ileocaecal junction.

Single or small groups (Fig. 1k) nerve cell bodies immunoreactive to PHI occurred in the submucosal plexus, in close association to submucosal blood vesels. PHI immunoractive ramified nerve fibres extended into the mucosa as well as to small blood vessels in the submucosa, while simple or ramified nerve fibres were seen in the circular smooth muscle layer at the ileocaecal junction. Small groups of nerve cell bodies immunoreactive to PHI were situated in the myenteric plexus.

Nerve cell bodies showing immunoreactivity to SP occurred singly or in groups in the submucosal plexus (Fig. 1f), and were often associated with submucosal blood vessels. Groups of nerve cell bodies immunoreactive to SP occurred in the myenteric plexus. Simple and ramified nerve fibres extended into the mucosa. Similar fibres were also seen associated with blood vessels in the submucosa. Ramified nerve fibres were demonstrated in the circular smooth muscle of the junctional area.

Controls for non-specific staining in this study were negative.

Discussion

At the ileocaecal junction, the peptides somatostatin, gastrin, glucagon and NPY, present in endocrine cells, could not be demonstrated in neurones. Conversely, immunoreactivity for VIP, SP, PHI and CGRP could only be detected in neurones in the gut wall and not in endocrinal cells.

The low concentration of somatostatin immunoreactive cells in the ileal part of the ileocaecal junction recorded in this study, corroborate the findings of Kitamura *et al.* (1984) in the ileum of the horse. However, the present study is the first to demonstrate the presence of somatostatin containing cells in the equine caecum. These findings are in line with previous authors who demonstrated somatostatin in the endocrine cells of the ileum and caecum of other domestic species; such as the sheep (Calingasan, Kitamura, Yamada, Oomori & Yamashita, 1984), the cat (Kitamura, Yamada, Yamashita & Yanaihara, 1982), the calf and occasionally in the adult bovine (Kitamura, Yamada, Calingasan, Yamashita, 1985) as well as in the ileum of the dog (Tange, 1983) and the pig (Ito, Yamada, Yamashita, Hashimoto & Kudo, 1987).

TABLE 3 The topographic distribution of neurones showing immunoreactivity to SP, VIP, PHI and CGRP respectively in the ileocaecal junction of the horse and the donkey

Antigen	Nerve cell bodies		Nerve fibres extending to:		
	Myenteric plexus	Submucosal plexus	Mucosa	Blood vessels	Circular smooth muscle
SP	√	√	√	√	√
VIP	√	√	√	√	√
PHI	√	√	√	√	√
CGRP	√	√	√	√	–

√ indicates immunoreactivity
– indicates no immunoreactivity

Somatostatin has been described mainly in laboratory animals to occur in endocrine cells of the gut mucosa, decreasing in number from the stomach to the colon, and in intrinsic neurons of the myenteric and submucosal plexuses respectively (Makhlouf, 1983). In this study somatostatin was found only in endocrine cells of the mucosa. According to Polak & Bloom (1983), somatostatin is a powerful inhibitory substance, which, depending on its topographic distribution, may act as a local hormone or a neurotransmitter. Considering the results of the present study, it is suggested that somatostatin may have a local hormonal function at the ileocaecal junction.

Immunoreactivity to gastrin in endocrine cells in the ileal and caecal parts of the ileocaecal junction, and the presence of glucagon immunoreactive cells in the ileal part of the junction found in this study, was not reported in the horse by Kitamura *et al.* (1985). However, gastrin immunoreactivity has been reported in the ileum of the sheep (Calingasan *et al.*, 1984) and the opossum (Krause, Yamada & Cutts, 1985). By decreasing the ileocaecal sphincter pressure, both synthetic and exogenous natural gastrins have an inhibitory effect on the ileocaecal junction in man (Castell, Cohen & Harris, 1970). Should gastrin have a similar effect in the horse, it may affect the closure of the ileocaecal orifice.

In this study endocrine cells showing immunoreactivity to glucagon were found in the ileal part of the junction only which is in contrast to the presence of glucagon in both the ileum and caecum of the sheep (Calingasan *et al.*, 1984), and in the cow and calf (Kitamura *et al.*, 1985). According to Ruppin and Domschke (1980), there are conflicting reports as to the function of glucagon in the different regions of the gut of several species. Nevertheless, it appears that glucagon has an inhibitory effect on gut motility. Should this be the case in the horse, glucagon may influence the closure of the ileocaecal orifice.

In this investigation, neurotensin immunoreactivity was found in the ileal part of the ileocaecal junction and underscores the findings of Kitamura *et al.* (1984), who detected neurotensin immunoreactivity in the ileum of the horse. The latter authors faled to detect neurotensin immunoreactivity in endocrine cells in the horse caecum. However, in the present study, cells immunoreactive to neurotensin were found in the caecal part of the ileocaecal junction. A high density of endocrine cells showing neurotensin immunoreactivity were recorded in the ileum and a lower concentration in the caecum of the dog (Tange, 1983), the ileum of the pig (Ito *et al.*, 1987), the cat (Kitamura *et al.*, 1982), the sheep (Calingasan *et al.*, 1984), and in the cow (Kitamura *et al.*, 1985).

Although neurotensin immunoreactivity was demonstrated only in endocrine cells in this study, it has been observed in the nerve fibres of various mammalian species (Reinecke, 1985). More recently, Buchan & Barber (1987) demonstrated significant amounts of neurotensin in the canine submucosal plexus and myenteric ganglia. Reinecke (1985) cited Hellström *et al.* (1982) to have found that neurotensin slowed down the passage of ingesta through the small intestine and that opening of the ileocaecal valve was suppressed by neurotensin.

The NPY immunoreactivity observed in some of the endocrine cells in the present study is unexpected, because this peptide is known to occur in neurones only (Solomon, 1985). As NPY shares amino acid sequences with the peptide YY (PYY) (Solomon, 1985), the antiserum to NPY may have cross-reacted with PYY in the gut endocrine cells in this study.

The presence of VIP- and SP immunoreactive neurones seen in this study coincides with the findings of Kitamura *et al.* (1984), who observed immunoreactivity to these peptides in the intestinal wall of the horse. In the present study, VIP, SP, CGRP and PHI immunoreactivity was often detected in nerve cell bodies and nerve fibres in close proximity to the blood vessels in the submucosa of the ileocaecal junction. VIP has a strong vasodilatory effect and is often found in close association with intestinal blood vessels (Furness & Costa, 1987).

CGRP immunoreactivity was observed in the gastrointestinal tract of the rat (Fujimura, Greely, Hancock, Alwmark, Santos, Cooper, Reumont, Ishizuka & Thompson, 1988) and other species (Rodrigo, Polak, Fernandez, Ghatei, Mulderry & Bloom, 1985). In this study, nerve cell bodies and nerve fibres showing immunoreactivity to CGRP were associated with submucosal blood vessels in particular. Their action on the submucosal venous plexus at the equine ileocaecal junction has not been determined. Although CGRP has been described as a potent vasodilator in other parts of the body, this function has not specifically been reported for intestinal blood vessels (Brain, Williams, Tippins, Morris & MacIntyre, 1985). Immunoreactivity to CGRP in the gastrointestinal tract has often been located in beaded nerve fibres associated with the smooth muscles of blood vessels (Rosenfeld, Mermond, Amara, Swanson, Sawchenko, Rivier, Vale & Evans, 1983), as was the case in this study.

In agreement with the present study, SP immunoreactivity has also been observed arround submucosal arterioles and veins in the human intestine (Llewellyn-Smith, Furness, Murphy, O'Brien & Costa, 1984). It has been suggested that SP and CGRP are stored together in the same secretory vesicles, released together from peripheral axons and that the effect of SP on blood vessels may be potentiated by CGRP (Gulbenkian, Merighi, Wharton, Varndell & Polak, 1986). Substance P has been shown to cause vasodilation of intestinal blood vessels and depolarization and contraction of intestinal smooth muscles (Furness & Costa 1987).

Even though VIP has a relaxant effect on the circular smooth muscles of sphincters (Goyal, Rattan & Said, 1980), no significant differences in concentrations of VIP in sphincter and non-sphincter regions in the intestine of the cat could be found (McGregor, Bishop, Blank, Christofides, Yiangou, Polak & Bloom, 1984).

Both PHI and VIP have been found to have a wide distribution in the intestinal tract of various species (Christofides, Polak & Bloom, 1984). In this study immunoreactivity to PHI was found in nerve fibres extending to the circular smooth muscle, to the submucosal blood vessels and into the mucosa. No specific data concerning the effect of PHI on intestinal smooth muscle or blood vessels have yet been reported. However, in general, the actions of this peptide seems to be similar to the actions of the glucagon-secretin family (Christofides *et al.*, 1984).

In this study, the presence of nerve fibres immunoreactive to VIP and SP which penetrated the muscularis mucosa and extended into the villi beneath the glandular epithelium, corresponds with the findings of Tange (1983) in the dog.

The musculature of the ileocaecal junction often differs from ileal and caecal musculature in its response to the same pharmacological agent (Cardwell, Rubin, Snape & Cohen, 1981). Endocrine cells showing immunoreactivity to somatostatin, gastrin, glucagon and NPY were very sparsely distributed in the present study. Furthermore, in the horse, the muscalature of the ileum decreases in thickness as the ileocaecal junction is reached (Kotzé, 1988a). Therefore, it appears that in the horse, these peptides play only a subsidiary role in regulating the function of the ileocaecal junction.

Of greater importance is the putative effect of SP, VIP, PHI and CGRP immunoreactive nerve fibres found in close association with the submucosal venous plexus of the equine ileocaecal junction (Kotzé, 1988b). The sympathetic nervous system is one of the primary factors that control the vascular resistance in the mesenteric vascular bed (Le Noble, Tangelder, Slaaf, Smits & Struyker-Boudier, 1987). However, the presence of these peptides in this area suggest that they may affect the engorgement of the plexus and thereby the function of the ileocaecal junction in the horse.

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