The organisation of the enteric nervous system in the submucous and mucous layers of the small intestine of the pig studied by VIP and neurofilament protein immunohistochemistry

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ABSTRACT

The arrangement of the enteric ganglia and nerve fibre plexuses was examined in the submucous and mucous layers and around Peyer’s patches of the porcine small intestine to clarify their organisation. Immunohistochemistry of vasoactive intestinal peptide (VIP) and neurofilament proteins in wholemounts, chopped or paraffin sections was used to locate the neural elements. The ganglia of the internal and external submucous plexuses were situated at 2 different topographic locations, being clearly demarcated by the submucosal vascular arcades and differing in neuronal composition. The internal submucous plexus was the only contributor to the plexus surrounding the follicles of Peyer’s patches as a continuous mesh of 3 ganglionated nerve subplexuses. VIP-immunoreactive fibres from this mesh innervated the dome. The mucosal plexus, which was subdivided into 4 subunits—the outer proprial, inner proprial, pericryptal and villous plexuses—contained a few solitary neuronal perikarya. Labelling for neurofilament proteins revealed Dogiel types II, IV and VI neurons. The observations reveal several new features in the enteric nervous system of the pig and clarify its nomenclature.

Key words: Small intestine; neurofilament protein; vasoactive intestinal peptide.

INTRODUCTION

In large animal species, the enteric nervous system (ENS) comprises 3 ganglionic plexuses: the myenteric plexus (MP), the internal submucous plexus (ISP) and the external submucous plexus (ESP) (Stach, 1988; Timmermans et al. 1992; Pearson, 1994). The ISP is also termed Meissner’s plexus and the ESP is called Henle’s or Schabadasch’s plexus (Scheuermann et al. 1987a, b). The glial cell mass is more abundant in the ESP than the ISP, and the ESP meshwork is wider than that of the ISP (Thomsen et al. 1997). In the ISP, numerous small ganglia are located at the intersections of the fibre mesh (Scheuermann et al. 1987a, b; Krammer & Kühnel, 1992). Neuropeptides show a distinct distribution between the 2 submucosal plexuses. Neuropeptide Y, somatostatin and enkephalin immunoreactive neuronal perikarya appear solely in the ESP, whereas those immunoreactive to neuromedin U are abundant in the ISP (Timmermans et al. 1990).

In view of these differences between the 2 submucosal plexuses in the small intestine of pig, Timmermans et al. (1990) proposed that they perform
different functions. This proposition has been supported by differences in electrophysiological properties between these plexuses as recorded by Pearson et al. (1996) and Thomsen et al. (1997).

Despite this information, there are contradictory descriptions of morphology and function between the submucous plexuses in the small intestine of the pig. For instance, there are reports of a third (intermediate) plexus in the submucous layer (Gunn, 1968). Scanning electron microscopy (SEM) shows that ISP ganglionic clusters and nerve fibre strands are situated at 2 topographic levels. The smallest ganglia are situated close to and associated with the lamina muscularis mucosae (Scheuermann et al. 1987a). In some respects, these observations support the existence of an intermediate plexus. In the small intestine of pig the ISP and ESP are separated by the submucosal vascular arcades with the ISP on the mucosal and the ESP on the serosal side (Scheuermann et al. 1987a, b; Thomsen et al. 1997). These arcades have been observed to underlie the lymphoid follicles (Lowden & Heath, 1994). In contrast, the EPS ganglia in Peyer’s patches have been reported to be situated close to the base of the lymphoid follicles and those of the ISP are in the interfollicular region (Krammer & Kühnel, 1993). Because of these contradictions we re-examined the morphological organisation of the ISP and ESP in the jejunum and ileum of pig.

According to Furness & Costa (1980), the mucous plexus is composed of fine nerve bundles and axons. The mucous plexus can be subdivided into subglandular, periglandular and villous components. Peyer’s patches play a central role in the uptake of antigens, induction of immune responses, proliferation of B lymphocytes and synthesis of immunoglobulin (Pabst, 1987). VIP modulates T-cell proliferation and regulates their function (Metwali et al. 1993). We have observed that VIP outlines organification of the ENS in the small intestine of pig.

Pooled antisera against neurofilament proteins (NF 200, 70 and 160 kDa) have been used to visualise the structure of the ENS in the sheep oesmus (Yamamoto et al. 1994). Antibodies against NF 200 kDa NF have also been used to visualise its organisation in the pig small intestine but reactivity to this antibody was only displayed in type II neurons (Krammer & Kühnel, 1992).

In the last 3 decades, the organisation of the ENS in the small intestine of pig has been studied mainly by histochemistry (Timmermans et al. 1990, 1992; Krammer & Kühnel, 1992) and SEM (Scheuermann et al. 1987a, b). The most recent study by conventional light microscopy is that of Mannl et al. (1986). These authors used toluidine blue and safranin staining to reveal topographic and neuronal differences between the 2 submucosal plexuses.

In this study, we have used histochemical staining for VIP- and NF-like immunoreactivity (IR) and histological staining by haematoxylin-eosin to elucidate the organisation of the ENS in the jejunum and ileum of pig. Emphasis has been placed on the organisation of the ENS in the tela submucosa, Peyer’s patches and tunica mucosa. We also aimed to describe clearcut criteria to differentiate the 2 submucosal plexuses and thereby establish an appropriate nomenclature.

MATERIALS AND METHODS

Sampling and fixation

Seven Danish Landrace/Yorkshire cross bred weaning piglets aged 6–8 wk (13–15 kg) fed on a standard commercial diet (N.A.G. Svine foder 5, Helsinge, Denmark) and 1 2-d-old piglet, were used. The 2 age groups were employed so as to compare the ease in microdissection. During the 12 h before anaesthesia, the piglets were allowed access to sterile drinking water containing d-glucose (55 g/l) only. Animals were sedated by an intramuscular injection of azaperone (5 mg/kg) (Stresnil, Jenssen Pharmaceutica, Belgium) 20 min before anaesthesia. This was induced by the intravenous injection of pentobarbitone sodium (10 mg/kg) and maintained by 2% halothane in oxygen via a semiclosed circuit.

A midline laparotomy was performed in the anaesthetised pigs and 2 (2–3 cm) pieces of intestinal tissue were obtained from the jejunum and ileum. Tissues were immersed in 0.01 M phosphate buffered saline (PBS, pH 7.3) in a Petri dish, opened along the mesenteric border and gently washed with PBS to remove faecal contents. They were pinned onto polystyrene and fixed by immersion in 4% formalin at room temperature. After 1 h, biopsies were transferred into fresh fixative after removal from the polystyrene and trimming 1 piece into a 1 cm × 0.5 cm block for embedding. Tissues for embedding were fixed for 7 d at 4°C; those for microdissection for 24 h at room temperature.

Embedding, sectioning and staining

Two 1 cm × 0.5 cm tissue blocks were dehydrated, cleared in xylene and embedded in paraffin wax. Two blocks of similar size were embedded in 5% molten Becto agar (50°C) and chopped into 100 µm pieces by
using a McIlwain tissue chopper (Mickle Laboratories, UK). Chopped sections were collected and processed as free floating sections in 0.01 M PBS + 0.5% Triton X-100 (washing buffer). They were washed in washing buffer while shaking on a HS 250 shaker (Janke and Kunkel, IKA, Labortechnik, Germany) at 200 cycles/min for 1 h. The washing buffer was changed every 20 min. Tissues were then kept in this buffer at 4 °C overnight.

Three 5 and 25 µm sections were cut from each paraffin block. The 5 µm sections were stained with haematoxylin-eosin-phloxine (HE). The 25 µm sections were collected in glass vials containing 0.5 ml xylene for clearing and subsequent rehydration. They were washed and thereafter kept in the washing buffer at 4 °C overnight.

**Microdissection**

The 1 cm × 2 cm formalin-fixed tissues were trimmed (1 cm × 1 cm), washed, and pinned stretched out in washing buffer on Sylgard (silicone rubber), mucosal surface uppermost, and viewed under a stereo microscope (Olympus, SZH. Japan; × 64 max. magn.). The mucosa was removed by scraping carefully using pair of blunt tissue forceps. Tissue wholemounts bearing the myenteric, internal and external submucous plexuses were teased apart using very fine tissue forceps and a scalpel blade to sever the tight interconnections (Pearson, 1994). Wholemount tissue was collected in 0.5 ml washing buffer contained in glass vials, washed and kept in the same buffer at 4 °C overnight.

**Immunohistochemical staining**

Wholemounts, deparaffinised (25 µm) and chopped (100 µm) free floating sections were rinsed in washing buffer for 20 min and then quenched against endogenous peroxidase reactivity using 3% aqueous hydrogen peroxide (Merck, Germany) for 20 min. Sections were immunolabelled overnight at 4 °C either with a rabbit anti-VIP (Fahrenkrug, Denmark) diluted 1:4000 or a mouse antihuman neurofilament protein (NF) (Sigma) antibody diluted 1:20. In both cases, a biotin-streptavidin-HRP revealing system was used. For VIP labelling, a biotinylated swine anti-rabbit antibody (Dako, Denmark) diluted 1:500 and for NF labelling a biotinylated rabbit antimouse antibody (Zymed, USA) at 1:500 were used. For the third layer, an avidin-biotin complex conjugated to HRP (Dako, Denmark) was used in both cases. DAB in 1:500 washing buffer was employed as a chromogen for 1 h without hydrogen peroxide, then 5–15 min with 0.01% hydrogen peroxide. All incubations were carried out in PBS with 0.5% Triton X-100 and at the blocking step and antibody incubations there was additional 5% nonimmune serum (swine or rabbit). Tissues from mouse brain and sheep omasum were used as positive controls for VIP and NF-like immunoactivities respectively to check reaction specificity. The reaction was stopped by rinsing tissues in distilled water for 30 min with the water being changed after every 10 min.

During each trial, half of the chopped tissues labelled for VIP were subdivided into 2 groups. One group was embedded in paraffin wax and another in Historesin (Leica, Heidelberg, Germany). These were sectioned and counterstained by HE.

**Tissue processing for mounting**

Wholemounts and chopped sections were dehydrated by graded passages in methanol followed by one step in isopropanol. They were then cleared in 1,2,3,4-tetrahydronapthalene (THN) (Aldrich, Germany) and thereafter in 20, 30 and 50% benzyl benzoate (Merck, Germany) in THN for 10 min at each step except the last in which they were left overnight. Paraffin sections were dehydrated in ethanol and brought to xylene. All tissues were mounted in DPX (Poole, UK).

**RESULTS**

**ENS organisation in the submucosa and tunica muscularis**

In the present study, 3 major plexuses of the ENS, namely the myenteric and external (ESP) and internal (ISP) submucous plexuses, were identified in sections and wholemounts (Figs 1a, b; 2a–d). Wholemounts and sections gave the same results, namely that ISP ganglia are situated at 2 different topographic levels, with the internal and external ISP ganglia being separated by a thin connective tissue layer (Fig. 1c). The ganglia and interganglionic nerve strands were intertwined and large interganglionic nerve strands and nerve fibres projecting into the submucous layer and lamina propria firmly attached the ISP to the lamina muscularis mucosae.

The ESP ganglia were also observed to be situated at 2 different topographic locations. They were seen to be situated a few micrometres from the inner circular smooth muscle layer of the tunica muscularis and occasionally very close to or between bundles of this muscle layer (Figs 1b, 4b). The connective
tissue around ESP ganglia was more dense when compared with that around those of the ISP. Connective tissue around the submucosal vascular arcades was less in comparison with the adjacent areas.

These observations imply that both the ISP and ESP ganglia are subdivided and situated at different topographic levels. However, ISP and ESP were clearly demarcated by the submucosal vascular arcades. The ISP was situated on the luminal and the ESP on the serosal side of these arcades. Wholemounts showed that the ESP meshwork is wider with larger ganglia, neurons and interconnecting nerve strands and thus fewer ganglia per unit area compared with ISP ganglia, which were abundant.

The perikarya with VIP-like IR were abundant in the inner submucous plexus, scarce in the outer submucous plexus and very infrequent in the
Fig. 2. VIP- and NF-like IR in wholemounts. (a) VIP-like IR in the ISP of a jejunal wholemount. The ISP ganglia (ISP) contain many VIP-like immunopositive neurons (arrows) and nerve fibre varicosities. Primary (interganglionic) nerve strands (ps) are larger but less wavy than secondary strands (ss). × 400. (b) Large myenteric plexus ganglion (MP) from an ileal wholemount. Shown are interganglionic nerve strands (ps), and many varicose nerve fibres (open arrow) which are also seen outside the ganglia as tertiary nerve strands. Nonimmunoreactive neurons (n). × 400. (c) Myenteric plexus ganglion (MP) from a jejunal wholemount showing NF-like IR. The inner circular muscle layer (IC) overlies the ganglion, whereas the outer longitudinal muscle underlies the ganglion. A positive nerve fibre (open arrow) can be identified in the circular muscle layer. Type IV neurons (arrowheads) are more abundant than type II neurons (arrow) which predominate in the ESP. × 400. (d) ESP ganglion (ESP) from a jejunal wholemount. Note NF-like IR Dogiel type II neurons (arrows), primary strands (ps), secondary strands (ss) and tertiary strands (ts). × 400.
myenteric plexus. The staining intensity in labelled perikarya decreased in the same order (Fig. 2a, b). The VIP-like IR nerve fibres were more abundant in the lamina propria and inner circular muscle than in the outer longitudinal muscle layer of the tunica muscularis.

Labelling for NF-like IR in strippings revealed clearly Dogiel type II, IV and VI neurons (Fig. 2c, d). However, it was not possible to correlate these to VIP-like IR neurons as double labelling was not done. In the ISP and ESP, neurons were intensely stained by VIP antibody and appeared to be Dogiel type II (Fig. 2a) neurons, whereas in the myenteric plexus, they showed weaker VIP immunoreactivity, and appeared to be different from those in the ISP and ESP.

**ENS organisation in Peyer’s patches**

Chopped, paraffin and Historesin sections revealed that in Peyer’s patches the ISP and ESP were separated...
Fig. 4. VIP-like IR at Peyer’s patches from the ileum. (a) Chopped section (100 µm) showing ISP ganglia (ISP) containing many positive neurons, under the base of the follicles (F) and in the corona (co). A dense mesh of positive fibres containing few immunopositive neurons (arrow) is seen in the corona (co) between the dome (d) and follicle proper (F). × 400. (b) HE stained 5 µm paraffin section, pig ileum. The ESP ganglion (ESP) in the ideal submucosa (SM) underlies a Peyer’s patches follicle (F) and is situated remote from the base of the follicle. This ganglion lies between muscle bundles of the inner circular muscle layer (IC). The ganglion has large neurons (arrow) and is situated below the level of the submucosal vascular arcades denoted by the vessel (v). The lamina propria (lp) is thin and has in an area been detached from the tela submucosa leaving an artefactual open space (x) under the follicle. × 840. (c) HE counterstained 2.5 µm section from a chopped tissue labelled for VIP before Historesin embedding and sectioning. VIP-like positive reactivity in the apex of the dome (d) is seen as nerve fibre varicosities (open arrow); e, dome epithelium. × 1690.

by the submucosal vascular arcades with the ESP being situated on the serosal side. The ISP was always seen on the luminal side of the arcades and formed a continuous mesh around the follicles of Peyer’s patches (Fig. 4a, b). This nerve meshwork appeared as 3 ganglionated plexuses, subdivisions of the ISP, around the follicles of Peyer’s patches. Of the ISP subdivisions, the first plexus was situated between the base of the follicles and the submucosal vascular arcades, the second in the interfollicular zone, and the third in the corona. Ganglia in these plexus subdivisions contained many VIP-like immunoreactive perikarya. The first 2 subdivisions had a wider mesh, larger ganglia and larger perikarya when compared with the third plexus. The latter was composed of smaller ganglia containing smaller perikarya and numerous fine nerve fibres forming a very dense nerve meshwork. VIP-like IR nerve fibres from the ISP meshwork innervated the dome but not the follicles proper (Fig. 4c). Observations that Peyer’s patches follicles are enclosed by ganglionated ISP meshwork were achieved by staining for VIP-like IR only.

**ENS in the lamina propria**

Labelling for VIP-like reaction product in chopped and paraffin sections revealed 4 distinct nerve plexuses formed by nerve fibres ascending into the lamina propria from the submucosa (Fig. 3a). We have applied simple terms to name these plexuses. The first 2 are the external propria plexus (EPP) (plexus lamina muscularis mucosae) which overlies the lamina mus-
Fig. 5. Schematic presentation (not to scale) of the identifiable plexuses in the ENS in the wall of the jejunum and ileum of the pig as revealed by VIP-like IR. The ENS in areas without and with Peyer’s patches follicles are shown by A and B respectively. The myenteric plexus (MP) is situated between the inner circular (ICM) and the outer longitudinal (OLM) muscle layers of the tunica muscularis. In the tela submucosa (SM) the ganglionated plexus is subdivided into the inner (ISP) and external submucosal (ESP) plexuses, this being demarcated by the submucosal vascular arcades (v). At the follicles of Peyer’s patches the ISP is subdivided into 3 subunits surrounding the follicles, seen at the base of the follicles (ISP1) (1), in traffic areas (ISP2) (2) and at the corona (ISP3) (3). The VIP-like IR neurons (dark profiles) are frequent in the ISP, infrequent in the ESP and very scarce in the myenteric plexus. Nonreactive neurons are denoted by open profiles. In the lamina propria, 4 plexuses, the external propria (I), the pericyptal (II), the inner propria (III) and the villus plexuses (IV) are shown in the tunica mucosa (M), where also a few solitary neurons (n) are seen. The tertiary aganglionic plexus (t) underlying the myenteric plexus and the subserosa plexus of the tunica serosa (S), although not discussed in the present work, is also visualised by staining for VIP-like IR.

The general organisation of the ENS in the small intestine of pig as revealed by staining for VIP is summarised in Figure 5.

**DISCUSSION**

**The ENS in the tela submucosa and tunica muscularis**

Our finding that the external (ESP) and internal (ISP) submucosal plexuses are topographically, structurally, and architecturally different is in agreement with the observations of Scheuermann et al. (1987a, b), Mannl et al. (1986) and Thomsen et al. (1997). Our findings using wholemounts and sections on ISP topography...
and its attachment to the lamina muscularis mucosae confirm those of Scheuermann et al. (1987 a, b), who used SEM on tissue wholemounts in contrast to our observations made in wholemounts stained by immunohistochemical methods and sections stained by HE. In conformity with Gunn (1968) we observed that the ISP ganglia of the pig were subdivided into external and internal ganglia; however, as we did not observe major morphological differences among the ISP ganglia at these 2 levels, we do not advocate the subdivision of this plexus.

Our findings that ESP ganglia are situated at 2 topographic levels support the results of Christensen & Rick (1987) and Hoyle & Burnstock (1989), who described them in the submucosa of the opposum and human colon, respectively. We however observed them to be in the submucosa of the pig jejunum and ileum.

The observation that the ISP and ESP are demarcated by the submucosal vascular arcades supports earlier findings by Scheuermann et al. (1987 a, b) and Thomsen et al. (1997). The submucosal vascular arcades are landmarks for topographic differences between the ISP and ESP, and the 2 plexuses can also be considered as different entities morphologically. The presence of thin bands of connective tissue in the area of the submucosal vascular arcades in comparison with adjacent areas is in agreement with the observations of Scheuermann et al. (1987a). This explains the ability to separate the tela submucosa by microdissection into ISP and ESP containing tissue laminae (wholemounts).

Our finding that VIP-like IR neurons were abundant in the ISP, infrequent in the ESP and very scarce in the myenteric plexus is in agreement with that of Timmermans et al. (1990). According to Scheuermann et al. (1987c), type II neurons are immunoreactive with antibodies to calcitonin gene related polypeptide (CGRP) and can be identified by their round, oval or triangular shaped cell bodies, with a smooth outline, from which long processes extend. The processes vary in calibre and divide into different branches at a fair distance from the perikaryon. Depending on the type of plexus, these neurons may contain substance P, VIP, galanin, and/or dynorphin (Timmermans et al. 1991). The abundance of VIP-IR nerve fibres in the ganglia, and the thickness of the wholemounts made it difficult to visualise the axons and dendrites to aid proper morphological identification. Nevertheless the VIP-like IR neurons appeared, from their shape, to be Dogiel type II neurons. In the myenteric plexus, VIP-containing neurons had the morphology of Dogiel type I, which receive cholinergic synaptic inputs (Costa et al. 1988). The VIP-IR neurons in the myenteric plexus were very sparse and stained less intensely. Whether they were Dogiel type I is uncertain but they appeared to be different from the neurons identified as Dogiel type II in the ISP and ESP.

According to Krammer & Kühnel (1992), NF (200 kDa)-like IR identifies adendritic, pseudounipolar to multiaxonal Dogiel type II neurons. The pooled NF antibody (200, 160 and 70 kDa), in addition, revealed the uniauxonal, multidendritic type IV and the uniauxonal multidendritic axodendritic type VI neurons. The NF 200 intermediate filaments are thought to exist in a high concentration and close arrangement in type II neurons and this is why antisera against the 200 kDa intermediate filaments ‘recognise’ type II neurons (Krammer & Kühnel, 1992). It may be considered that binding to type IV and VI neurons was displayed either by 70 or 160 kDa or both types of antineurofilament protein antibodies and that these neurons have higher concentrations either of 70 or 160 kDa, or both types of intermediate filaments.

The observed variation in the number of neurons as revealed by labelling for both VIP- and NF-like immunoreactivity supports the finding by Timmermans et al. (1990) that the submucous plexuses differ in neurochemical and neuronal contents. The fact that immunoreactivity to pooled antisera was not detected in all perikarya is in agreement with the finding by Björklund et al. (1984) and Krammer & Kühnel (1992) that, in the gastrointestinal tract, a small number of ganglion cells cannot be stained by antibodies to neurofilament protein.

**ENS organisation in Peyer’s patches**

According to Krammer & Kühnel (1993), the ESP is situated close to the base of the follicles in the Peyer’s patches of the small intestine of pig. Our results by labelling for VIP-like antigens and HE staining revealed that ISP surrounds follicles of Peyer’s patches as a continuous mesh of the 3 subdivisions (subplexuses) of the ISP nerve meshwork. This is in accordance with the general view that Peyer’s patches intrude into the submucosa and therefore will most likely be closely related to the ISP. The differences between our observations and those of Krammer & Kühnel (1993) could be due to the markers (protein gene product 9.5, neuron-specific enolase, neurofilament 200, S-100 protein and the glial fibrillary acidic protein) used by Krammer & Kühnel. They could not demonstrate neurons in the third ISP subdivision in Peyer’s patches. Therefore there is a
need to use other ENS markers to study this complexity.

In Peyer’s patches, nerve fibres are found in the dome but not in the follicle proper (Krammer & Kühnel, 1993). The VIP-like IR nerve fibres were also detected in the dome only. This indicates that a particular subset of lymphocytes in the dome is innervated by VIP immunoreactive fibres. The functional significance of this observation requires identification of cells specifically innervated by these fibres.

The ENS in the tunica mucosa

Our results on VIP-like IR showed that nerve fibre meshes in lamina propria form 4 distinct nerve plexuses. We propose for them the following nomenclature: (1) the external propria plexus (EPP), (2) the internal propria plexus (IPP); (3) the pericryptal plexus (PCP) and (4) the villus plexus (VP). According to Furness & Costa (1980) and Costa & Brookes (1994), the ENS in the mucosa plexus is composed of fine nerve bundles and axons and can be subdivided into subglandular, periglandular and the villous components. Except for the description of the IPP which is new, our findings are consistent with their description of the EPP, PCP and VP.

We detected the presence of solitary neuronal perikarya in the porcine pericryptal plexus. These perikarya are immunoreactive to antisera in staining for VIP-like proteins and give rise to positively stained varicose nerve fibres extending towards both serosal and luminal surfaces. Probably they act as integration centres of impulses in the mucosal reflexes which do not involve relay neurons of the submucosal and myenteric plexuses.

The fact that we did not observe similar cells in sections labelled with NF-like antisera may be due to poor penetration or that the antibody was unable to bind to filaments in these cells.

The observation of abundant VIP-like IR varicose nerve fibres close to the luminal epithelium and goblet cells signifies the importance of VIP in regulating epithelial transport and possibly to stimulate secretion in goblet cells.

Conclusions

Our results have shown that in the pig jejunum and ileum the ESP is subdivided at 2 different topographic levels, that the pericryptal plexus contains solitary neuronal perikarya and that a ‘new plexus’, the internal propria plexus, is well developed. In the areas of Peyer’s patches our results show that it is the inner submucosal plexus, ISP, alone, that contributes to the plexus surrounding the follicles and that it is subdivided into 3 compartments.

The controversy over the names of submucosa plexuses (Scheuermann et al. 1987b; Hoyle & Burnstock 1989; Pearson, 1994) has been clarified and we have used the most appropriate descriptive terminology to name the plexuses in the submucosa and lamina propria as indicated above and schematised in Figure 5.

Further studies using supplementary histochemical staining combined with laser scanning confocal microscopy might contribute to elucidation of fine details of the innervation and organisation of the enteric nervous system in the small intestine of the pig and other animals.

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