Anat Embryol (2003) 207:101–108
DOI 10.1007/s00429-003-0342-5

ORIGINAL ARTICLE

Nicola Mirabella · Caterina Squillacioti · Angelo Genovese · Giuseppe Germano · Giuseppe Paino

Topography and neurochemistry of the enteric ganglia in the proventriculus of the duck (*Anas platyrhynchos*)

Accepted: 14 July 2003 / Published online: 20 August 2003

Abstract The topographical distribution of the enteric ganglia has been investigated in the proventriculus of the duck using protein gene product 9.5 (PGP 9.5) immunohistochemistry. Myenteric ganglia were usually located between the outer longitudinal and the inner circular muscle layer. Submucous ganglia were sparsely distributed and seemed to be substituted by ganglia located in the tunica mucosa. The neurochemical profile of proventricular ganglion cells was also investigated using nicotinamide adenine dinucleotide phosphate reduced-diaphorase (NADPH-d)-histochemistry and pituitary adenylate cyclase activating peptide (PACAP)/galanin (Gal) double-labelling immunohistochemistry. The majority of mucosal ganglion cells were shown to contain the NADPH-d enzyme and both the investigated peptides. These findings provide evidence for the presence of a mucosal ganglionated plexus in the glandular stomach of birds. Moreover, the neurochemical characteristics of this plexus suggest that it plays an important role in regulating several mucosal functions and, in particular, the production and the composition of the gastric juice.

Keywords Muscularis mucosae · Proventricular glands · Gastric secretion · NO · Galanin

Introduction

The stomach of birds is divided into two portions: the proventriculus or glandular stomach, which produces the gastric juices, and the gizzard or muscular stomach which grinds the ingested food.

The anatomical structure of the proventriculus is characterized by the presence of two gland types: the superficial tubular and the deep compound proventricular glands (Chodnik 1947; Calhoun 1954; Farner 1960; Hodges 1974). Diverging opinions exist among the authors regarding the position of the deep proventricular glands and the layering of the tunica muscularis and muscularis mucosae. Some authors consider these glands to be located in the tela submucosa, the tunica muscularis being 3-layered with bands of longitudinal muscle located inside and outside a circular band (Bradley and Grahame 1960), or double-layered with outer circular and inner longitudinal bands (Martìnez et al. 1991). According to this organization, the lamina muscularis mucosae is formed by small, diffuse bundles of longitudinal fibres lying internal to the deep proventricular glands. Conversely, other authors (Calhoun 1954; Farner 1960) consider the deep proventricular glands to be located in the mucosal lamina propria. The tunica muscularis is composed of outer longitudinal and inner circular layers and the inner strand of longitudinal muscle is identified, in this case, as the outer portion of the lamina muscularis mucosae. This latter arrangement is that accepted by the Nomina Anatomica Avium (McLelland 1993) which reports that the muscularis mucosae is split by the deep proventricular glands into a inner and outer layer.

Despite the controversy existing about the layering of the proventricular wall and the position of the deep proventricular glands, little attention has been paid to the topographical distribution of the proventricular enteric ganglia.

It is commonly accepted that in birds, as in mammals, the enteric ganglia form two ganglionated plexuses, i.e. the myenteric and submucous plexuses which are located between the inner circular and outer longitudinal layers of the tunica muscularis and in the tela submucosa, respectively (Ali and McLelland 1978, 1979, 1980). Bird enteric ganglion cells, moreover, have been extensively reported to contain neuropeptides and the enzyme NADPH-diaphorase (Vittoria et al. 1992; Boros et al. 1994; Li et al. 1994; Balaskas et al. 1995; Suzuki et al. 1996; Mirabella et al. 2000, 2002). In the proventriculus, the distribution and neurochemistry of enteric ganglia have been studied in a few species of birds. Immunopositivity

N. Mirabella (✉) · C. Squillacioti · A. Genovese · G. Germano · G. Paino
Department of Structures Functions and Biological Technologies, Faculty of Veterinary Medicine,
Via Veterinaria, 80137 Naples, Italy
e-mail: mirabell@unina.it

to vasoactive intestinal peptide (VIP) and/or the enzyme NOS has been described in proventricular myenteric and submucous ganglion cells of the chicken (Balaskas et al. 1995; Martìnez et al. 2000), quail (Li et al. 1994) and raptor bird houbara bustard (Mensah-Brown and Lawrence 2001). However,, in the proventriculus of the duck, the majority of neuropeptide-containing enteric ganglion cells have been reported to be located in the tunica mucosa (Mirabella et al. 2002).

The main purpose of the present study was, therefore, to clarify the topographical distribution of the avian proventricular enteric ganglia. Moreover, in order to improve the knowledge about the role played by nerves in regulating proventricular activity, the neurochemical profile of proventricular ganglion cells was investigated using histochemistry and double-labelling immunohistochemistry.

Material and methods

Adult Campbell khaki ducks (*Anas platyrhynchos*) of both sexes were used. They were anesthetized by intramuscular injection of ketamine (25 mg/kg), sacrificed by exanguination and specimens of proventriculus were collected.

Tissue preparation

Frozen sections

The specimens were fixed in 4% paraformaldehyde in a 0.1 M phosphate-buffered saline (PBS) solution at pH 7.5 for 2–3 h and placed successively in PBS containing 0.1% sodium azide and 10% sucrose and stored overnight at 4°C. The following day, the samples were transferred to a mixture of PBS-sucrose-azide and OCT compound (Tissue Tek, Elkhart, IN) in a ratio of 1:1 for 24 h before being embedded in 100% OCT. Coronal sections of 10–20 μm thickness were cut.

Paraffin-embedded sections

The specimens were fixed by immersion in Bouin's fixative for 12 h and stored in 70% ethanol. The pieces were dehydrated through a graded ethanol series and embedded in paraffin wax. Coronal sections of 6 μm thickness were cut. Several paraffin-embedded sections were stained with haematoxilin-eosin.

Immunohistochemistry and colocalization studies

The paraffin-embedded sections were treated by the peroxidase antiperoxidase (PAP) method (Sternberger 1979) using a primary antibody against PGP 9.5 (rabbit, dilution 1:1000, cod. 7863-0504, Biogenesis, Poole, UK).

The frozen sections were immunostained according to the fluorescent technique. They were preincubated in 10% normal goat serum (NGS) (X0907, Dako A/S, Glostrup, Denmark) in PBS containing 0.1% Triton X-100 (PBS-T) overnight at 4°C. They were then rinsed 3×10 min in PBS-T prior to the exposure to the primary antisera. Antisera against pituitary adenylate cyclase polypeptide (PACAP)-38 (rabbit, dilution 1:200, cod. IHC 8920, Peninsula Laboratories) and anti-galanin (Gal) (rabbit, dilution 1:200, cod. IHC 7153, Peninsula Laboratories) were used for single and double labelling techniques. After an overnight incubation at 4°C, excess serum was washed off with 3×10 min changes in PBS-T. The sections were then incubated for 1 h at room temperature with an appropriate rhodamine (TRITC) or fluorescein (FITC)-conjugated secondary antibody, goat-anti-guinea pig rhodamine (TRITC) conjugated antibody (cod. AQ 108 R) and goat-anti-rabbit fluorescein (FITC) conjugated antibody (cod. AQ 132 F, Chemicon International). After washing 3×10 min in PBS-T, the sections were mounted in 1:1 PBS-glycerol and then examined and photographed.

The specificity of primary antibodies was tested by preabsorption of the primary antibody with excess of the appropriate antigens. The specificity of the immunoreactions was tested by omitting the primary antibody, using buffer instead. No immunoreaction was detected in control tests. For double labelling the sections were incubated in a 1:1 mixture of primary antisera. Each antiserum had a final dilution as in the single labelling. After washing 3×10 min in PBS-T, the sections were incubated in a 1:1 mixture of secondary antibodies with an initial dilution of 1:50. The secondary antibodies and the incubation times were the same as those used for single labelling. To ensure that no cross-reactivity of secondary detection system occurred, the primary antisera were alternatively omitted in control tests. No cross-reactivity was observed.

NADPH-diaphorase histochemistry

The frozen sections were hydrated in distilled water, immersed in several changes of Tris-HCl buffer 0.1 M pH 7.8 (TBS) and incubated in NADPH-incubating medium in the dark at 37°C for 1 h. The incubating medium was composed of TBS containing 0.2 mg/ml nitroblue tetrazolium (N 6876, Sigma-Aldrich, Milan, Italy), 2.7 mg/ml l-malic acid (M 9138, Sigma), 1 mg/ml α-NADPH (N 7505, Sigma-Aldrich, Milan, Italy) and 0.2% Triton X-100. After incubation, the sections were rinsed in three changes of TBS, mounted in PBS/glycerol (1:1) and then examined and photographed.

Results

The wall of the duck proventriculus appeared, from the outside to the inside, to be composed of the following layers (Fig. 1a, b): tunica serosa; stratum longitudinale of the tunica muscularis, stratum circulare of the tunica muscularis, tela submucosa, tunica mucosa. The lamina muscularis mucosae was formed by outer and inner layers which, in turn, enclosed the deep proventricular glands. The outer layer of the lamina muscularis mucosae was dense and had a longitudinal direction, whereas, the inner layer was composed of sparse muscle fibre bundles having an irregular direction. According to these findings, the deep proventricular glands were located in the tunica mucosa.

Almost all the neurons contained in the enteric ganglia were stained by PGP 9.5 immunohistochemistry. Myenteric ganglia were usually located between the outer longitudinal and the inner circular muscle layer (Fig. 2a). Occasionally, large positive ganglia were found in a subserosal position. Positive cells were very rarely observed in the tela submucosa, where they were isolated or formed small groups of 2–3 elements. Many PGP 9.5 positive ganglia were found in the tunica mucosa, the majority of which were located between the inner side of the outer layer of the muscularis mucosae and the basis of the deep proventricular glands (Fig. 2b). Small groups or single cells were, in addition, found in the interglandular

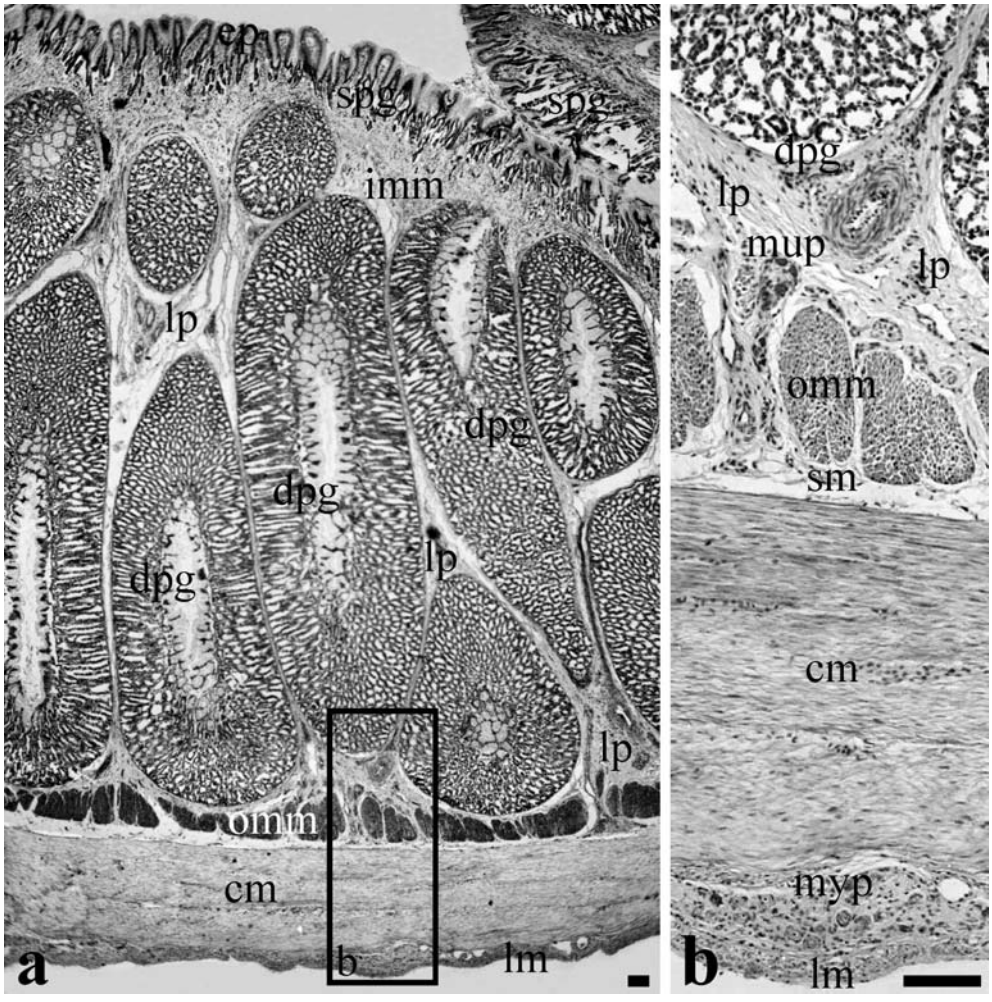

Fig. 1a, b Histological organization of the wall of the proventriculus (*lm* longitudinal muscle layer, *cm* circular muscle layer, *sm* tela submucosa, *omm* outer layer of the muscularis mucosae, *dpg* deep proventricular glands, *lp* lamina propria, *imm* inner layer of the muscularis mucosae, *spg* superficial proventricular glands, *ep* lining epithelium of the proventricular mucosa, *myp* mienteric plexus, *mup* mucosal plexus). The deep proventricular glands were located between the outer and inner layers of the tunica muscularis mucosae (**a**). The tunica muscularis was formed by outer longitudinal and inner circular layers (**b**). H&E staining, *bar*=50 μm

septa between the deep proventricular glands (Fig. 2c) and beneath the proventricular plicae where the superficial proventricular glands are located (Fig. 2d). Frequently, the continuity of the outer layer of the lamina muscularis mucosae was observed to be interrupted by spaces containing ganglia and nerve bundles (Fig. 2e,f).

NADPH-diaphorase histochemistry showed similar results to those obtained by PGP 9.5 immunohistochemistry. The majority of the cells contained in myenteric (Fig. 3a) and mucosal (Fig. 3c–f) ganglia were positively stained. Few, isolated positive stained cells were found in the tela submucosa (Fig. 2b). Positive nerve fibres were observed to innervate primarily the circular layer of the tunica muscularis and blood vessels. The alveolar epithelium of the deep proventricular glands also stained positively.

The distribution of the PACAP immunoreactivity in the duck proventriculus has been previously described (Mirabella et al. 2002). Double immunostaining for PACAP and Gal revealed coexistence of these two neuropeptides in many enteric ganglionic cells and in nerve fibres. The majority of PACAP/Gal positive cells were found in the mucosal enteric ganglia (Fig. 4a–d). PACAP/Gal positive nerve fibres were found to innervate the circular and longitudinal layers of the tunica muscularis. In addition, these fibres richly innervated the tunica mucosa. In particular, they innervated the muscularis mucosae (Fig. 4a–d), the proventricular plicae (Fig. 4e,f) and the ductal epithelium of the deep proventricular glands (Fig. 4g,h).

Discussion

The presence of few ganglionic or single neurons in the lamina propria of the gastrointestinal mucosa has been well documented in several mammalian species (Drasch 1881; Vau 1932; Stöhr 1934, 1944; Lassmann 1975; Newson et al. 1979; Mestres et al. 1992a, 1992b; Balemba et al. 1999; Wedel et al. 1999). These neurons have been also found to contain neuropeptides (Schultzeberg et al. 1980; Korman et al. 1989; Vittoria et al. 1992; Bredkjaer et al. 1994; Balemba et al. 1998) and the NADPH-d enzyme (Fang et al. 1993). Neverthless, mucosal ganglia and single neurons are considered to be ectopic from the submucous plexus (Stöhr 1934; Lassmann 1975) and the mucous plexus to be aganglionic (Schultzeberg et al. 1980; Costa et al. 1987; Timmermans et al. 1990; Burns

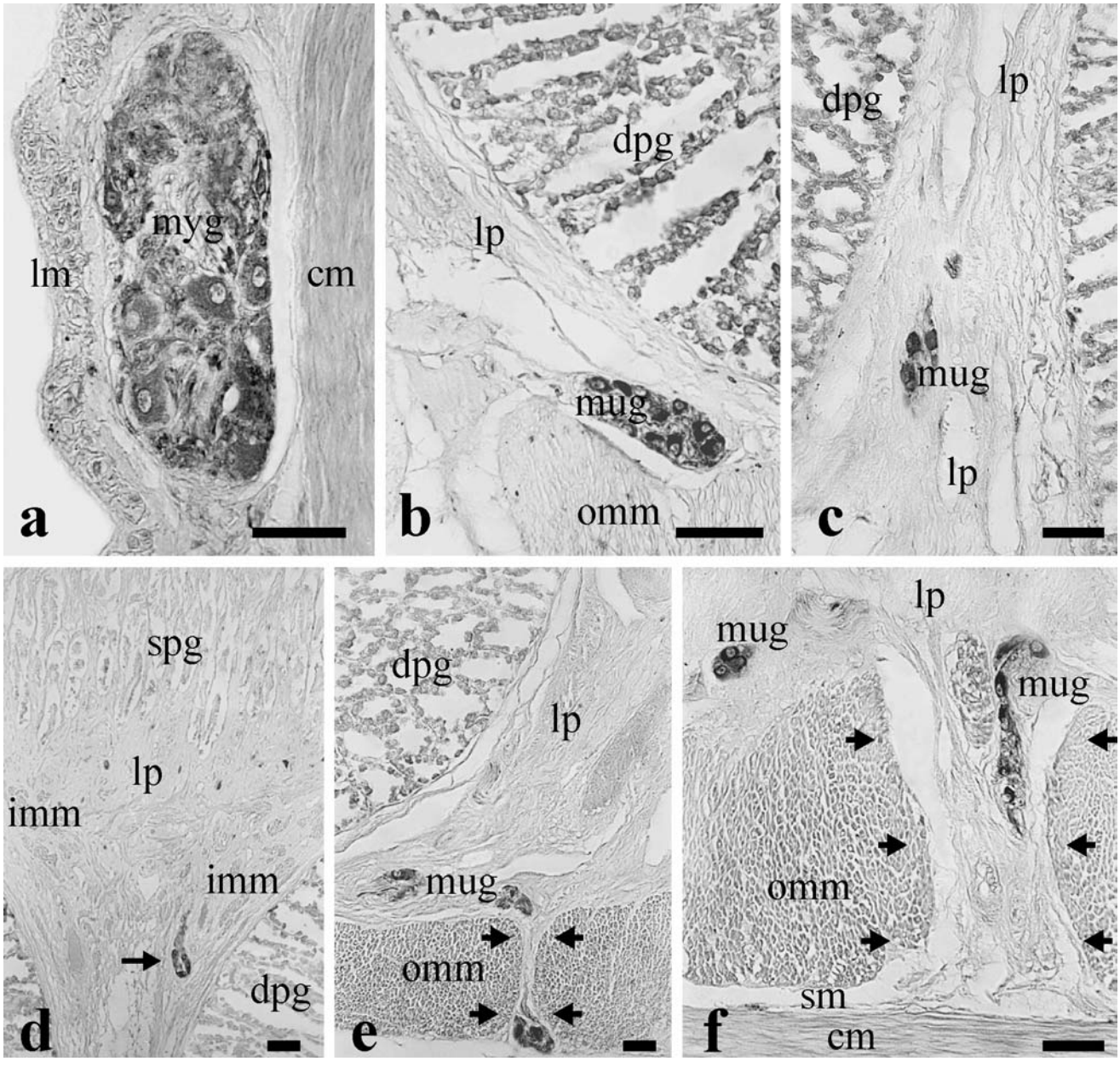

Fig. 2a–f Distribution of PGP 9.5 immunoreactivity (*lm* longitudinal muscle layer, *cm* circular muscle layer, *myg* myenteric ganglion, *omm* outer layer of the muscularis mucosae, *mug* mucosal ganglia, *lp* lamina propria, *dpg* deep proventricular glands, *imm* inner layer of the muscularis mucosae, *spg* superficial proventricular glands, *sm* tela submucosa). Myenteric ganglia were located between the outer longitudinal and the inner muscular layer of the tunica muscularis **a.** Mucosal ganglia were located at the base of deep proventricular glands **b** and within the interglandular septa **c**. Positive nerve cells (*arrow*) were also located in the lamina propria underneath the superficial proventricular glands **d**. Frequently, spaces containing ganglia and nerves **e, f** were observed to interrupt the continuity of the outer layer of the muscularis mucosae. *Bar*=25 μm

and Cummings 1993). Recently, however, the presence of a ganglionated plexus has been described in the tunica mucosa of the porcine intestine (Balemba et al. 2002).

The results of the present study have shown that submucous ganglia are sparcely distributed in the proventriculus of the duck and substituted by ganglia located in the tunica mucosa. Up to now, however, the enteric nervous system in the avian proventriculus has been considered to be regularly formed by myenteric and submucous ganglionated plexuses and no presence of ganglia or single neurons has ever been described in the tunica mucosa (Li et al. 1994; Balaskas et al. 1995; Martìnez et al. 2000; Mensah-Brown and Lawrence 2001). The reason for this controversy may reside in the fact that the histological organization of the proventriculus has been given different interpretations. The deep proventricular glands have been considered to be located in the tela submucosa and, most unusually, the tunica

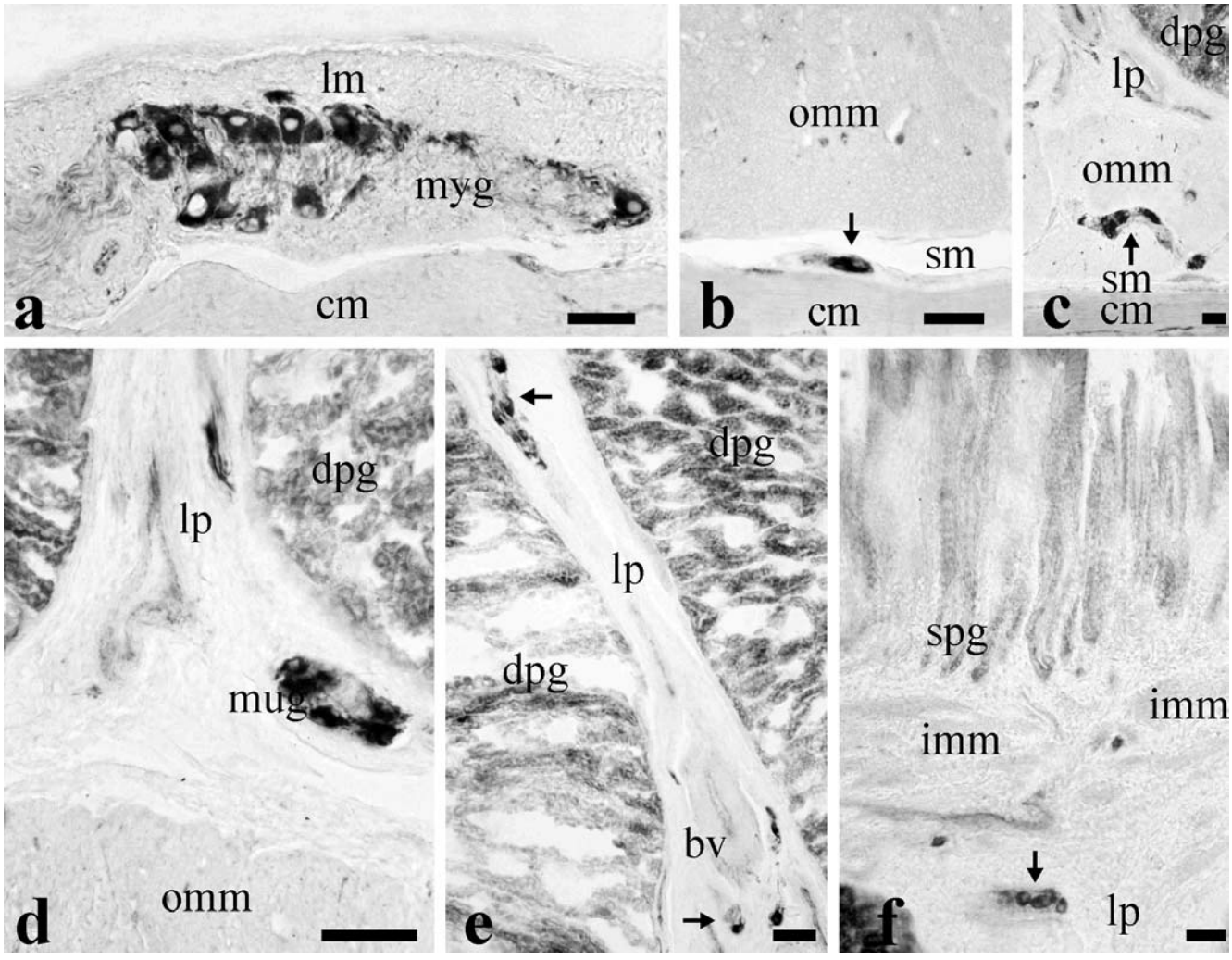

Fig. 3a–f Distribution of NADPH-d histochemistry (*lm* longitudinal muscle layer, *cm* circular muscle layer, *myg* myenteric ganglion, *omm* outer layer of the muscularis mucosae, *sm* tela submucosa, *mug* mucosal ganglin, *lp* lamina propria, *dpg* deep proventricular glands, *bv* blood vessels *imm* inner layer of the muscularis mucosae, *spg* superficial proventricular glands, *arrows* NADPH-d-positive nerve cells). Many NADPH-d positive nerve cells were found in myenteric **a** and mucous **d** ganglia. Groups of positive cells were also found in the outer layer of the muscularis mucosae **c**, in the interglandular septa **e** and underneath the superficial proventricular glands. Single positive cells were rarely found in the tela submucosa **b**. *Bar*=25 μm

muscularis to be 3-layered or, alternatively, composed of outer circular and inner longitudinal layers. In the present study, we have considered the deep proventricular glands to be located in the tunica mucosa and the lamina muscularis mucosae to be divided into inner and outer layers. Accordingly, the tunica muscularis was formed by the usual thick, inner, circular and a much thinner, outer, longitudinal layer. This histological organization, which is that given in the NAA (McLelland 1993), implies a topographical distribution of the enteric ganglia that differs from the pattern of distribution usually occurring in other sections of the avian (Ali and McLelland 1978, 1979, 1980) and mammalian (Furness and Costa 1987; Gershon et al. 1994) alimentary canal: proventricular enteric ganglia are virtually absent in the tela submucosa but form a true ganglionated plexus in the tunica mucosa. It can be hypothesized that submucous ganglia are displaced from the tela submucosa to the tunica mucosa during the development. The ganglia would follow the deep proventricular glands while these glands penetrate the muscularis mucosae and separate it into inner and outer layers (Farner 1960). The occurrence of spaces which interrupt the continuity of the outer layer of the muscularis mucosae supports this hypothesis. These spaces may indeed serve as passages for the migration of the glands, vessels and nerve structures from the tela submucosa to the tunica mucosa.

NADPH-d histochemistry has demonstrated that nitric oxide (NO) producing neurons are widely distributed in myenteric as well as mucosal ganglia of the duck proventriculus. The presence of NO-producing neurons has been previously reported in the proventriculus of several other avian species (Boros et al. 1994; Li et al. 1994; Balaskas et al. 1995; Martinez et al. 2000; Mensah-

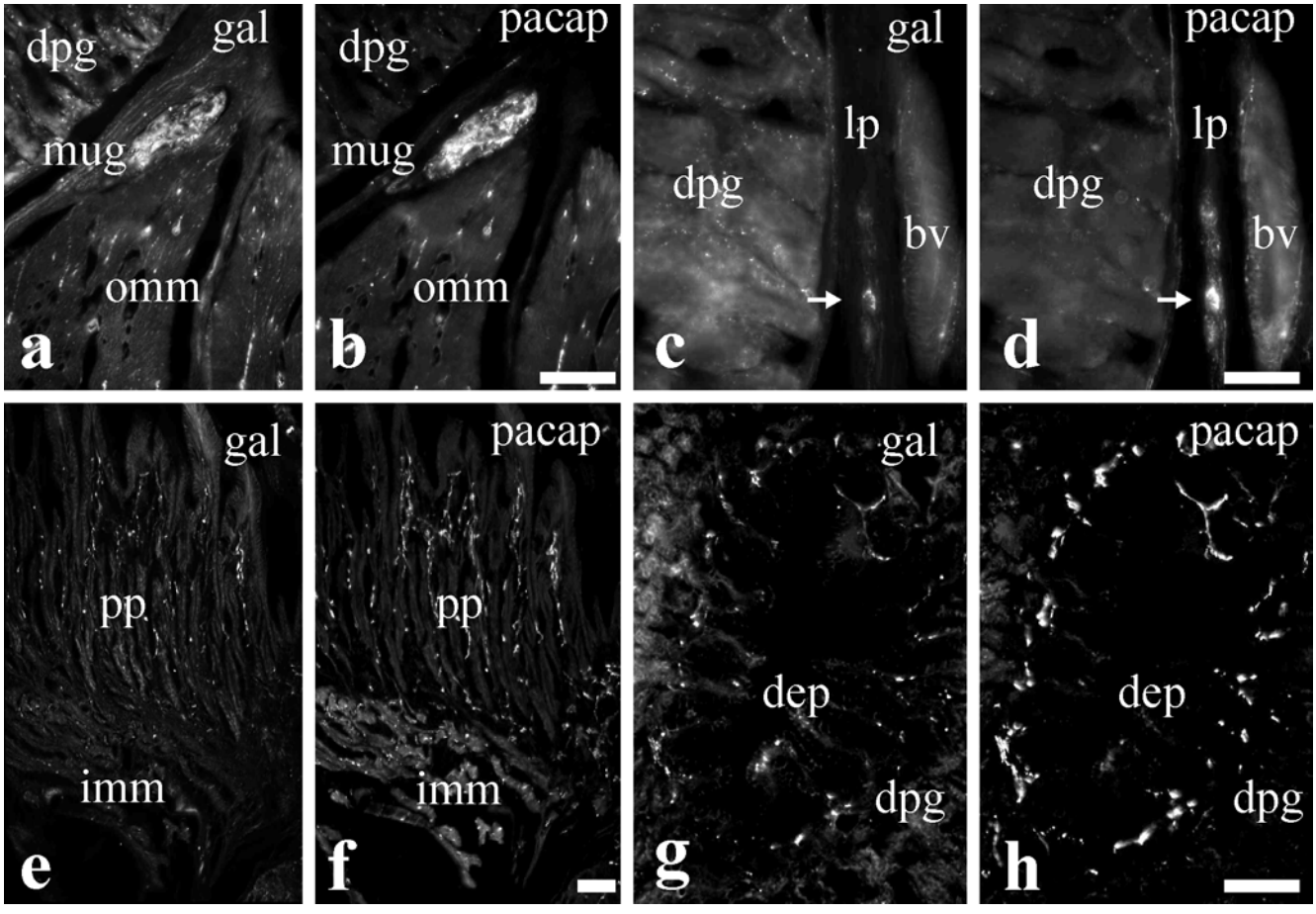

Fig. 4a–h Gal/PACAP colocalization studies. Gal (*gal*) and PACAP (*pacap*) were colocalized in neurons **a–d** and nerve fibres **e–h** innervating the proventricular mucosa. Gal/PACAP-positive cell bodies were found in ganglia located **a, b** at the base of the deep proventricular glands and **c, d** within the interglandular septa. Gal/PACAP positive fibres innervated the outer layer of the muscularis mucosae **a, b**, the proventricular plicae **e, f** and the duct epithelium in the deep proventricular glands **g, h** (*omm* outer layer of the muscularis mucosae, *mug* mucosal ganglia, *dpg* deep proventricular glands, *lp* lamina propria, *bv* blood vessels, *pp* proventricular plicae, *imm* inner layer of the muscularis mucosae, *dep* epithelium of the deep proventricular gland ducts, *arrow* a Gal/PACAP positive mucous nerve cell, *bar* 25 μm

Brown and Lawrence 2001) as well as in the mammalian stomach (Ekblad et al. 1994a, 1994b; Furness et al. 1994; Shemann et al. 1995; Jarvinen et al. 1999; Vittoria et al. 2000; Miller et al. 2001; Peng et al. 2001). NO has been shown to be involved in the non-adrenergic, non-cholinergic (NANC) inhibitory neurotransmission regulating the adaptive relaxation of the stomach (Desai et al. 1991; Zheng et al. 1999). Moreover, NO has been found to be an important messenger in the gastric mucosa. It mediates or modulates several physiological activities such as vasodilation, acid and bicarbonate secretion. In particular, NO seems to function as a mediator responsible for both stimulation, via histamine released from enterochromaffin-like (ECL) cells, and inhibition, via inhibition of parietal cell function, of gastric acid secretion (Yokotani et al. 1997; Hasebe et al. 1998, 2001; Kato et al. 1998a).

The immunohistochemical findings of the present study have shown that Gal is widely expressed in both proventricular ganglion cells and nerve fibres. In particular, Gal-ir is distributed in neurons and nerve fibres innervating the tunica mucosa. A widespread Gal-ir has been also reported in proventricular mucosal nerve fibres in the houbara bustard (Mensah-Brown and Lawrence 2001). Conversely, no Gal-ir has been observed in the chicken proventriculus (Martinez et al. 2000) and, generally, Gal-ir is little expressed in the mucosa of the mammalian stomach (Ekblad et al. 2000). The action of Gal on the gastrointestinal smooth musculature is controversial. Gal has been found to have an inhibitory role in vivo in man (Bauer et al. 1989) and, conversely, an excitatory role in vitro in rats (Ekblad et al. 1985), pigs (Botella et al. 1992) and mice (Fontaine and Lebrun, 1989). Both the responses, i.e., inhibition and stimulation, have been described in guinea pigs and dogs probably on the basis of the existence of different types of Gal-binding receptors and of their location (King et al. 1989; Botella et al. 1995). Gal, moreover, has been ascribed to inhibit acid gastric secretion by suppression of histamine release from ECL cells (Lindström et al. 1997; Kato et al. 1998b; Lindström and Håkanson 2001).

Double labelling immunohistochemistry disclosed a high degree of colocalization of Gal and PACAP in nerve cells and fibres innervating the proventricular mucosa. Since PACAP has been found to colocalize with the enzyme nitric oxide synthase (NOS) throughout the duck gastrointestinal tract (Mirabella et al. 2002), at least a part of the mucosal nerve cells expressing Gal/PACAP should also contain NOS. It should be remembered that PACAP potently stimulates gastric acid secretion by eliciting histamine release from ECL cells (Lindström et al. 1997; Lindström and Håkanson 2001). Thus, in the duck proventriculus, the same mucosal nerve cell may release several messengers both excitatory and inhibitory of the gastric acid secretive function.

In conclusion, the findings of the present study provide evidence for the presence of a mucosal ganglionated plexus in the glandular stomach of birds. The morphological and neurochemical characteristics of this plexus suggest that it plays an important role in regulating several mucosal functions and, in particular, the production and the composition of the gastric juice.

References

Ali HA, McLelland J (1978) Avian enteric nerve plexuses. A histochemical study. Cell Tissue Res 189:537–548

Ali HA, McLelland J (1979) Neuron number in the intestinal myenteric plexus of the domestic fowl (*Gallus gallus*). Anat Histol Embryol 8:277–283

Ali HA, McLelland J (1980) Variations in neuron size in the avian intestinal myenteric plexus. Anat Anz 147:348–353

Balaskas C, Saffrey MJ, Burnstock G (1995) Distribution and colocalization of NADPH-diaphorase activity, nitric oxide synthase immunoreactivity, and VIP immunoreactivity in the newly hatched chicken gut. Anat Rec 243:10–18

Balemba OB, Grøndahl ML, Mbassa GK, Semuguruka WD, Hay-Schmidt A, Skadhauge E, Dantzer V (1998) Organization of the enteric nervous system in the submucous and mucous layers of the small intestine of the pig studied by VIP and neurofilament proteins immunohistochemistry. J Anat 192:257–267

Balemba OB, MBassa GK, Semuguruka WD, Assey RJ, Kahwa CKB, Hay-Schmidt A, Dantzer V (1999) The topography, architecture and structure of the enteric nervous system in the jejunum and ileum of cattle. J Anat 195:1–9

Balemba OB, Hay-Schmidt A, Assey RJ, Kahwa CKB, Semuguruka WD, Dantzer V (2002) An immunohistochemical study of the organization of ganglia and nerve fibres in the mucosa of the porcine intestine. Anat Histol Embryol 31:237–246

Bauer FE, Zintel A, Kenny MJ, Calder D, Ghatei MA, Bloom SR (1989) Inhibitory effect of galanin on postprandial gastrointestinal motility and gut hormone release in humans. Gastroenterology 97:260–264

Boros A, Timmermans JP, Fekete E, Adriaensen D, Scheuermann DW (1994) Appearance and some neurochemical features of nitrergic neurons in the developing quail digestive tract. Histochemistry 101:365–374

Botella A, Delvaux M, Bueno L, Frexinos J (1992) Intracellular pathway triggered by galanin to induce contraction of pig ileum smooth muscle cells. J Physiol 458:475–486

Botella A, Jeanneton O, Delvaux M, Frexinos J, Bueno L (1995) Iloprost: intracellular Ca^{2+}-dependent contractile effect on isolated smooth muscle cells from guinea-pig ileum. J Pharm Pharmacol 47:398–401

Bradley OC, Grahame T (1960) The structure of the fowl, 4th edn. Oliver and Boyd, Edinburgh

Bredkjaer HE, Wulff BS, Emson PC, Fahrenkrug J (1994) Location of PHM/VIP mRNA in human gastrointestinal tract detected by in situ hybridisation. Cell Tissue Res 276:229–238

Burns GA, Cummings JF (1993) Neuropeptide distributions in the colon, caecum, and jejunum of the horse. Anat Rec 236:341–350

Calhoun ML (1954) Microscopic anatomy of the digestive system of the chicken. Iowa State College Press, Ames, Iowa

Chodnik KS (1947) A cytological study of the alimentary tract of the domestic fowl (*Gallus domesticus*). Q J l Microsc Sci 88:419–443

Costa M, Furness JB, Llewellyn-Smith IJ (1987) Histochemistry of the enteric nervous system. In: Jonson LR, Christensen J, Jackson MJ, Jacobson ED, Walsh JH (eds) Physiology of the gastrointestinal tract. Raven Press, New York, pp 1–39

Desai KM, Sessa WC, Vane JR (1991) Involvement of nitric oxide in the reflex relaxation of the stomach to accomodate food or fluid. Nature 351:477–479

Drasch O (1881) Beiträge zur Kenntniss des feineren Baues des Dünndarms, insbesondere über die Nerven desselben. Sitzber Akad Wiss Wien 82, 3rd div:168–198

Ekblad E, Rokaeus A, Hakanson R, Sundler F (1985) Galanin nerve fibers in the rat gut: distribution, origin and projections. Neuroscience 16:355–363

Ekblad E, Alm P, Sundler F (1994a) Distribution, origin and projections of nitric oxide synthase-containing neurons in gut and pancreas. Neuroscience 63:233–248

Ekblad E, Mulder H, Eddmann R, Sundler F (1994b) NOS-containing neurons in the rat gut and coeliac ganglia. Neuropharmacology 33:1323–1331

Ekblad E, Mei Q, Sundler F (2000) Innervation of the gastric mucosa. Microsc Res Tech 48:241–257

Fang SY, Wu RW, Christensen J (1993) Intramucosal nerve cells in human small intestine. J Auton Nerv Syst 44:129–136

Farner DS (1960) Digestion and the digestive system. In: Marshall AJ (ed) Biology and comparative physiology of birds, vol I. Academic Press, New York

Fontaine J, Lebrun P (1989) Galanin: Ca^{2+}-dependent contractile effects on isolated mouse distal colon. Eur J Pharmacol 164:583–586

Furness JB, Costa M (1987) The enteric nervous system. Churchill Livingstone, Edinburgh

Furness JB, Li ZS, Young HM, Forstermann U (1994) Nitric oxide synthase in the enteric nervous system of the guinea pig: a quantitative description. Cell Tissue Res 277:139–149

Gershon MD, Kirchgessner AL, Wade PR (1994) Functional anatomy of the enteric nervous system. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Raven Press, New York, pp 381–422

Hasebe K, Horie S, Yano S, Watanabe K (1998) Inhibitory effect of N^{ω}-nitro-L-arginine on gastric secretion induced by secretagogues and vagal stimulation in the isolated stomach. Eur J Pharmacol 350:229–236

Hasebe K, Horie S, Komasaka M, Yano S, Watanabe K (2001) Stimulatory effects of nitric oxide donors on gastric acid secretion in isolated mouse stomach. Eur J Pharmacol 420:159–164

Hodges RD (1974) The histology of the fowl. Academic Press, New York

Jarvinen MK, Wollmann WJ, Powrozek TA, Schultz JA, Powley TL (1999) Nitric oxide synthase-containing neurons in the myenteric plexus of the rat gastrointestinal tract: distribution and regional density. Anat Embryol 199:99–112

Kato S, Kitamura M, Korolkiewicz RP, Takeuchi K (1998a) Role of nitric oxide in regulation of gastric acid secretion in rats: effects of NO donors and NO synthase inhibitor. Br J Pharmacol 123:839–846

Kato S, Korolkiewicz R, Rekowski P, Szyk A, Sugawa Y, Takeuchi K (1998b) Inhibition of gastric acid secretion by galanin in rats. Relation to endogenous histamine release. Regul Pept 74:53–59

King SC, Slater P, Turnberg LA (1989) Autoradiographic localization of binding sites for galanin and VIP in small intestine. Peptides 10:313–317

Korman LY, Sayid H, Bass B, Moody TW, Harmon JW (1989) Distribution of vasoactive intestinal polypeptide and substance P receptors in human colon and small intestine. Dig Dis Sci 34:1100–1108

Lassmann G (1975) Vorkommen von Ganglienzellen im Schleimhautstroma von Colon, Sigma und Rectum. Virchows Arch A Pathol Anat Histol 365:257–261

Li ZS, Young HM, Furness JB (1994) Nitric oxide synthase in neurons of the gastrointestinal tract of an avian species, *Coturnix coturnix*. J Anat 184:261–272

Lindström E, Håkanson R (2001) Neurohormonal regulation of secretion from isolated rat stomach ECL cells: a critical reappraisal. Regul Pept 97:169–180

Lindström E, Björkqvist M, Boketoft Å, Chen D, Zhao CM, Kimura K, Håkanson R (1997) Neurohormonal regulation of histamine and pancreastatin secretion from isolated rat stomach ECL cells. Regul Pept 71:73–86

Martìnez A, Lòpez J, Barreneches MA, Sesma P (1991) Immunocytochemical and ultrastructural characterization of endocrine cells in chicken proventriculus. Cell Tissue Res 263:541–548

Martìnez A, Lòpez J, Sesma P (2000) The nervous system of the chicken proventriculus: an immunocytochemical and ultrastructural study. Histochem J 32:63–70

McLelland J (1993) Apparatus digestorius (Systema alimentarium). In: Baumel JJ, King AS, Breazile JE, Evans HE, Vanden Berge JC (eds) Handbook of avian anatomy: nomina anatomica avium. The Nuttal Ornithological Club, Cambridge, Massachusetts, pp 301–327

Mensah-Brown EPK, Lawrence PA (2001) Neurotransmitters regulating acid secretion in the proventriculus of the Houbara bustard (Chlamydotis undulata): a morphological viewpoint. J Morphol 248:175–184

Mestres P, Diener M, Rummel W (1992a) Electron microscopy of the mucosal plexus of the rat colon. Acta Anat 143:275–282

Mestres P, Diener M, Rummel W (1992b) Histo- and immunocytochemical characterization of the neurons of the mucosal plexus in the rat colon. Acta Anat 143:268–274

Miller SM, Reed D, Sarr MG, Farrugia G, Szurszewski JH (2001) Haem oxygenase in enteric nervous system of human stomach and jejunum and colocalization with nitric oxide synthase. Neurogastroenterol Motil 13:121–131

Mirabella N, Lamanna C, Assisi L, Botte V, Cecio A (2000) The relationships of nicotinamide adenine dinucleotide phosphate-d to nitric oxide synthase, vasoactive intestinal polypeptide, galanin and pituitary adenylate activating polypeptide in pigeon gut neurons. Neurosci Lett 293:147–151

Mirabella N, Squillacioti C, Colitti M, Germano G, Pelagalli A, Paino G (2002) Pituitary adenylate cyclase activating peptide (PACAP) immunoreactivity and mRNA expression in the duck gastrointestinal tract. Cell Tissue Res 308:347–359

Newson B, Ahlman H, Dahlström A, Das Gupta TK, Nyhus LM (1979) Are there sensory neurons in the mucosa of the mammalian gut? Acta Physiol Scand 105:521–523

Peng X, Feng JB, Yan H, Zhao Y, Wang SL (2001) Distribution of nitric oxide synthase in stomach myenteric plexus of rats. World J Gastroenterol 7:852–854

Schultzeberg M, Hökfelt T, Nilsson G et al. (1980) Distribution of peptide- and catecholamine-containing neurons in the gastrointestinal tract of rat and guinea-pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine α-hydroxylase. Neuroscience 5:689–744

Shemann M, Schaaf C, Mader M (1995) Neurochemical coding of the enteric neurons in the guinea pig stomach. J Comp Neurol 353:161–178

Sternberger LA (1979) Immunocytochemistry. Wiley, New York

Stöhr P (1934) Mikroskopische Studien zur Innervation des Magen-Darmkanales III. Z Zellforsch Mikrosk Anat 21:243–278

Stöhr P (1949) Mikroskopische Studien zur Innervation des Magen-Darmkanales V. Z Zellforsch Mikrosk Anat 34:1–37

Suzuki M, Ohmori Y, Watanabe T (1996) Immunohistochemical studies on the intramural nerve in the chicken intestine. Eur J Histochem 40:109–118

Timmermans JP, Scheurmann DW, Stach W, Adriaensen D, Groodt-Lasseel MHA de (1990) Distinct distribution of CGRP-, enkephalin-, somatostatin-, substance P-, VIP- and serotonin-containing neurons in the two submucosal ganglionic neural networks of the porcine small intestine. Cell Tissue Res 260:367–379

Vau E (1932) Über die subglandulären Ganglienzelle in der Magenwand einiger Haussäugetiere. Anat Anz 73:380–385

Vittoria A, Castaldo L, La Mura E, Lucini C, Cecio A (1992) VIP-immunoreactive nerve structures of the gastrointestinal tract in the developing and adult domestic duck. Arch Histol Cytol 55:361–374

Vittoria A, Costagliola A, Carrese E, Mayer B, Cecio A (2000) Nitric oxide-containing neurons in the bovine gut, with special reference to their relationship with VIP anf galanin. Arch Histol Cytol 63:357–368

Wedel T, Roblick U, Gleiss J, Schiedeck T, Bruch HP, Kühnel W, Krammer HJ (1999) Organisation of the enteric nervous system in the human colon demonstrated by wholemount immunohistochemistry with special reference to the submucous plexus. Anat Anz 181:327–337

Yokotani K, Murakami Y, Okuma Y, Osumi Y (1997) Centrally applied nitric oxide donors inhibit vagally evoked rat gastric acid secretion: involvement of sympathetic outflow. Jpn J Pharmacol 74:337–340

Zheng ZL, Rogers RC, Travagli RA (1999) Selective gastric projections of nitric oxide synthase-containing vagal brainstem neurons. Neuroscience 90:685–694