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THE NEUROENDOCRINE SYSTEM OF THE GUT—AN UPDATE

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Abstract

During the last few years the endocrine stomach has come into focus much due to the side-effects produced by powerful acid blockers. A sustained and marked inhibition of acid secretion in the rat results in hypergastrinemia, with gastrin cell hyperplasia, and a consequent hyperplasia of the ECL cells. This response of the ECL cells was predictable in view of previous observations that sustained hypergastrinemia causes ECL cell hyperplasia. While the gastrin cell hyperplasia levels off at about twice the normal cell density a few weeks after start of treatment, the ECL cells continue to proliferate for months to reach a five-fold higher density than normally. Evidence is accumulating that ECL cells proliferate through self replication. After life-long inhibition of acid production (high doses of ranitidine or omeprazole) or after extirpation of 75% of the acid-producing part of the stomach, ECL cell carcinoids develop. Endocrine cells in the gut often contain more than one putative messenger. Thus, gastrin cells in many species store GABA and peptide YY; in e.g. cat and man they store in addition a xenopsin-like peptide. Neuromedin U and pituitary adenylate cyclase activating peptide (PACAP) have recently been demonstrated in gut nerves. Their role in gut physiology remains to be identified.

Key words: Gut endocrine cells, gastrin cells, ECL cells, peptide hormones, neuropeptides, peptide coexistence, neuromedin U, PACAP.

The gastrointestinal tract harbours a well developed neuroendocrine system comprising numerous peptide hormone-producing endocrine cells and a rich autonomic innervation. Many regulatory peptides have been demonstrated in endocrine cells and/or in nerves and their number is still increasing. Despite the progress made in the cellular localization of putative gut hormones there are still many endocrine cells where a peptide hormone is anticipated to exist but has not yet been identified. The oxyntic mucosa of the stomach, for instance, harbours several endocrine cell types that do not contain an identified peptide hormone (1, 2).

The last few years have witnessed a growing interest in the endocrine stomach. This is partly because new and powerful blockers of acid secretion, such as proton pump inhibitors and unsurmountable H_2-antagonists, were found to have dramatic effects on the endocrine stomach (2).

A summary of recent findings related to the gastric endocrine cells is given below followed by an update on the gut innervation, with special reference to newly described neuropeptides.

The endocrine stomach

The two major populations of endocrine cells in the stomach are the gastrin cells in the antrum and the enterochromaffin-like (ECL) cells in the oxyntic mucosa (1). The gastrin cells are sensitive to stimuli from the lumen (nutrients and pH) and pharmacological blockade of acid secretion is associated with an increased gastrin cell activity with hypergastrinemia as a consequence. If the blockade is marked and long-lasting, the plasma gastrin levels may increase several-fold, remaining elevated as long as the blockade persists (3–6). Studies in the rat have revealed that after a few weeks of acid blockade the number of gastrin cells has increased by 50–100% (7, 8). After this initial rapid rise in gastrin cell density the cell number remains stable despite maintained acid blockade (9). Even after one year of acid blockade the gastrin cell density remains at the level seen after a few weeks (Mattsson et al., to be published). Possibly, counter-regulatory forces might play a role in preventing progressive gastrin cell hyperplasia after the initial steep rise in cell number. This is

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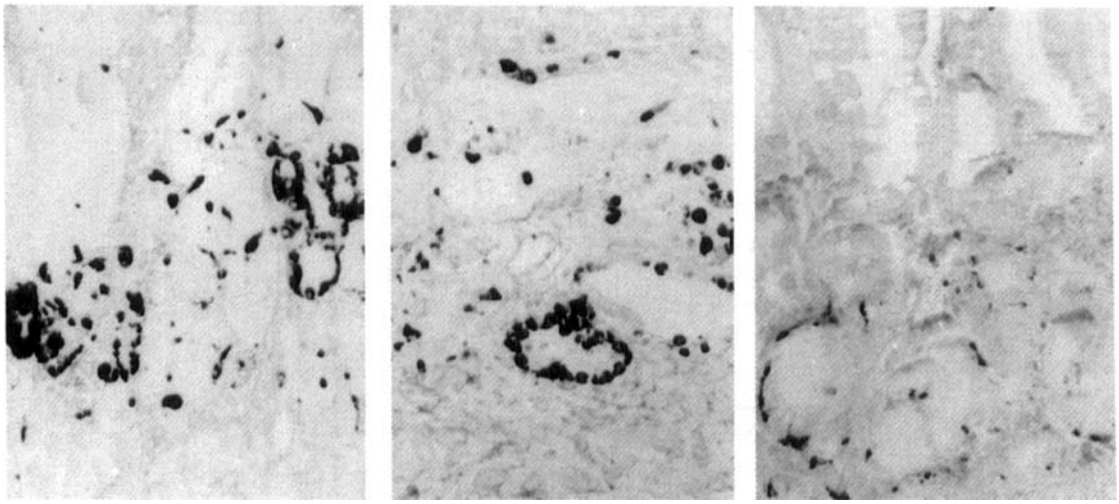
reflected in the fact that the ^{3}H-thymidine labelling index of the gastrin cells, i.e. the proportion of cells synthesizing DNA, drops to control levels after an initial increase (9).

One of the consequences of a long-term, sustained blockade of acid secretion is ECL cell hyperplasia (3–6) and, after life-long treatment with acid blockers, formation of ECL cell tumours. Much evidence suggests that gastrin plays a major role in mediating this effect (the gastrin hypothesis) (2). Nevertheless, there has been a reluctance to accept the hypothesis (10, 11). The fact that the ECL cell hyperplasia induced by acid blockers can be mimicked by continuous infusion of exogenous gastrin (12), seems to support the gastrin hypothesis.

Upon activation, the ECL cells behave somewhat differently from the gastrin cells. In the rat, prolonged stimulation of the ECL cells results in a steadily increasing cell density for up to at least 20 weeks (5). The ^{3}H-thymidine labelling index increases steadily for about ten days and remains elevated (9, 13, 14). The plasma gastrin level and ECL cell labelling index seem to be well correlated; this has been shown in experiments in the rat (13–16). There is strong evidence that the ECL cells divide and that they multiply by self replication (13–16). As mentioned above acid blockade over a long period of time may result in the formation of ECL cell carcinoids. This was first shown in the rat after life-long treatment with proton pump inhibitors, such as omeprazole (17), and with so-called unsurmountable H_2-antagonists (18). More recently, resection of 75% of the acid-producing mucosa in rats was shown to be sufficient to cause gastrin cell activation and hyperplasia, hypergastrinemia, and ECL cell hyperplasia in the remaining oxyntic mucosa (19). At the end of a two-year study ECL cell carcinoids developed (20). Very recently the well-known H_2 blocker ranitidine, given to rats in high doses mixed with the food, was also found to cause marked hypergastrinemia, ECL cell hyperplasia and, in the end, ECL cell carcinoids (21), much in the same way as the proton pump inhibitors and the unsurmountable H_2-blockers. Since in the ranitidine-fed rats plasma gastrin levels were greatly elevated only during the night, i.e. the feeding period for the rat (22), these data indicate that the plasma gastrin levels may not have to be elevated permanently to induce ECL cell hyperplasia and ECL cell carcinoids. Nevertheless, accumulating evidence suggest that carcinoid tumours of the oxyntic mucosa are gastrin-dependent neoplasms (23–26). It is therefore possible, even likely, that removal of the antrum, the main store of endogenous gastrin, could interfere with the development and growth of gastric carcinoids. In fact, there are several reports suggesting that the ECL cell hyperplasia, the micronodules and possibly also the carcinoids in these patients disappear after antrectomy (27–31) (Fig. 1). Correspondingly, we have observed a marked reduction in the ECL cell density after removal of the gastrin-producing tumour in a patient with the Zollinger-Ellison syndrome (unpublished observations).

The ECL cells in the rat are known to store large amounts of histamine. With the availability of histamine antibodies, immunocytochemistry could be used to reveal the presence of histamine in the ECL cells also of other aminal species (32) and man (32, 33). There are, however, marked variations among species as to the relative proportion of the two major stores of histamine in the oxyntic mucosa: ECL cells and mast cells. In the rat, mast cells are few and about 90% of the mucosal histamine resides in ECL cells. In man, on the other hand, mucosal mast cells are numerous and rich in histamine, while the ECL cells are relatively few in number and display a weak histamine



a) b) c)

Fig. 1. Oxyntic mucosa of a patient with chronic atrophic gastritis, hypergastrinemia and multiple gastric carcinoids. Specimens taken a) before, b) 6 months, and c) 16 months after antrectomy. Sections immunostained for chromogranin A to demonstrate endocrine cells. Before antrectomy there was a severe hyperplasia of endocrine (mostly ECL) cells. The hyperplasia was slightly less severe 6 months after antrectomy. Not until 16 months after the operation were the endocrine cells markedly reduced in number (and size). (× 150).

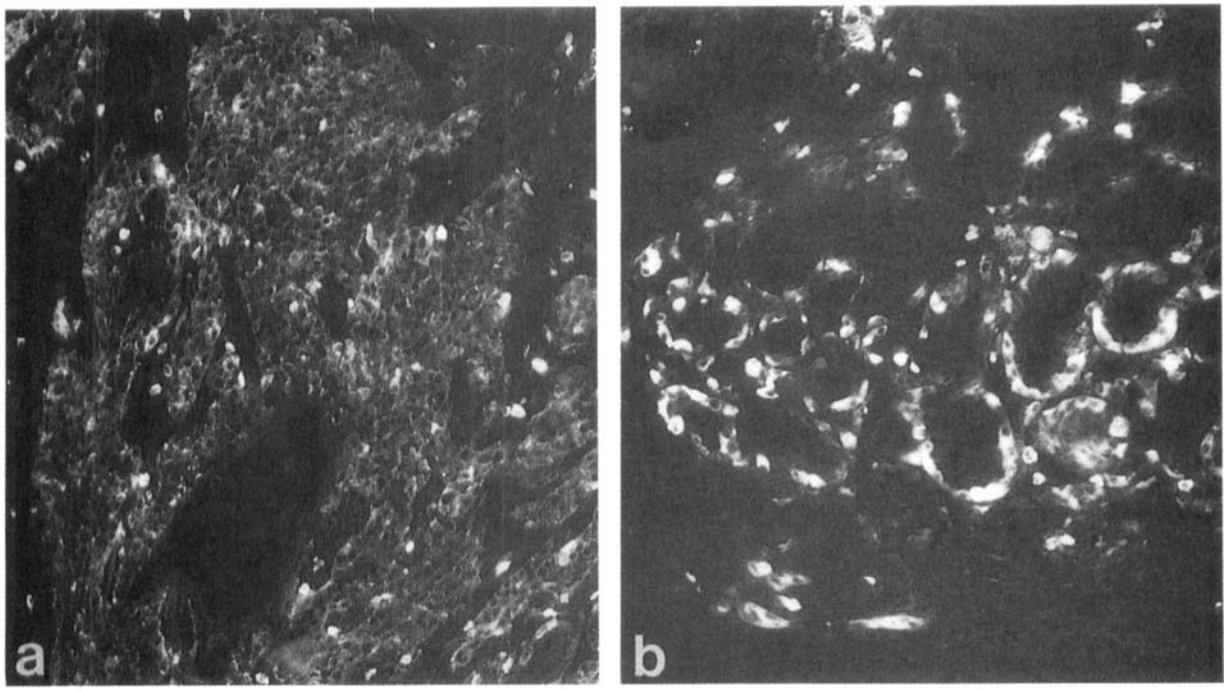


Fig. 2. a) Gastric carcinoid and b) non-tumorous oxyntic mucosa of a patient with chronic atrophic gastritis. Sections immunostained for histamine. The tumour cells and the hyperplastic ECL cells in the non-tumorous mucosa display histamine immunofluorescence. (× 200).

Fig. 3. Human oxyntic mucosa. Section immunostained for the calcium binding protein calbindin. Calbindin immunofluorescence is present in scattered cells, many of which issue cytoplasmic processes. The cells are identical with ECL cells (c.f. ref. 39). (× 250).

immunostaining. Thus in the human oxyntic mucosa most histamine resides in mast cells. The histamine-producing capacity of the ECL cells is retained in tumours arising from ECL cells (ECL cell carcinoids or ECL-omas) (Sundler et al., to be published) (Fig. 2).

Gastrin is capable of inducing hypocalcemia in the rat without affecting the release of calcitonin (34–36) and to enhance the uptake of calcium into bone (37). These effects are prevented by gastrectomy or by removal of the acid-producing part of the stomach indicating that the factor mediating the gastrin response resides in the oxyntic mucosa (37, 38). An extract of the oxyntic mucosa has blood-calcium-lowering activity and the active agent appears to be a peptide (38). Since the blood-calcium-lowering factor is released by gastrin, it is tempting to suggest that it resides in the ECL cells. It may be mentioned in this context that the ECL cells seem to be the only cells in the human oxyntic mucosa that contain the calcium-binding protein calbindin (39) (Fig. 3).

Coexistence of putative messengers in gastric endocrine cells

The gastrin cells in the antrum produce not only gastrins, but also other regulatory peptides as demonstrated by immunocytochemistry. Thus, the antral gastrin cells in several mammals store immunoreactive PYY (40–42). In some species all the gastrin cells seem to contain PYY. In

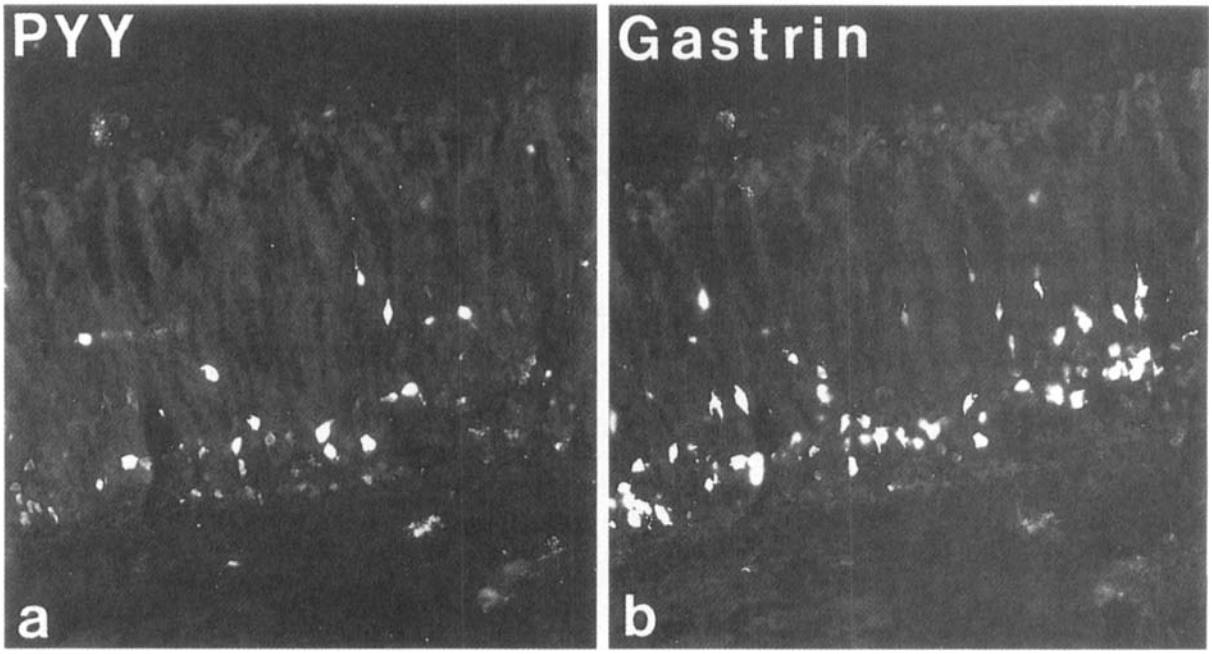


Fig. 4. Rat antral mucosa. Cryostat section processed for the simultaneous demonstration of a) PYY and b) gastrin using double immunostaining. The PYY-immunofluorescent cells constitute a subpopulation of the gastrin cells. (× 150).

other species PYY is stored in a subpopulation of the gastrin cells (unpublished observations) (Fig. 4). In the rat, the PYY-immunoreactive gastrin cells are rather few in adult animals but quite numerous in early life (41). Interestingly, as studied in the rat, the PYY-immunoreactive cells in the antrum also contain immunoreactive GABA (Fig. 5). GABA has recently been reported to occur in endocrine cells of the rat stomach (43, 44) and in the rat antrum immunoreactive GABA seems to reside not only in gastrin cells, but also in a subpopulation of somatostatin cells. In dog and man, xenopsin-like immunoreactivity occurs in the antral gastrin cells (45, 46) (Fig. 6). Xenopsin is a neurotensin-related peptide first isolated from the skin of the frog Xenopus laevis. In the chicken antrum the gastrin cells are rich in neurotensin (47). Pancreastatin, a putative regulatory peptide arising from chromogranin A

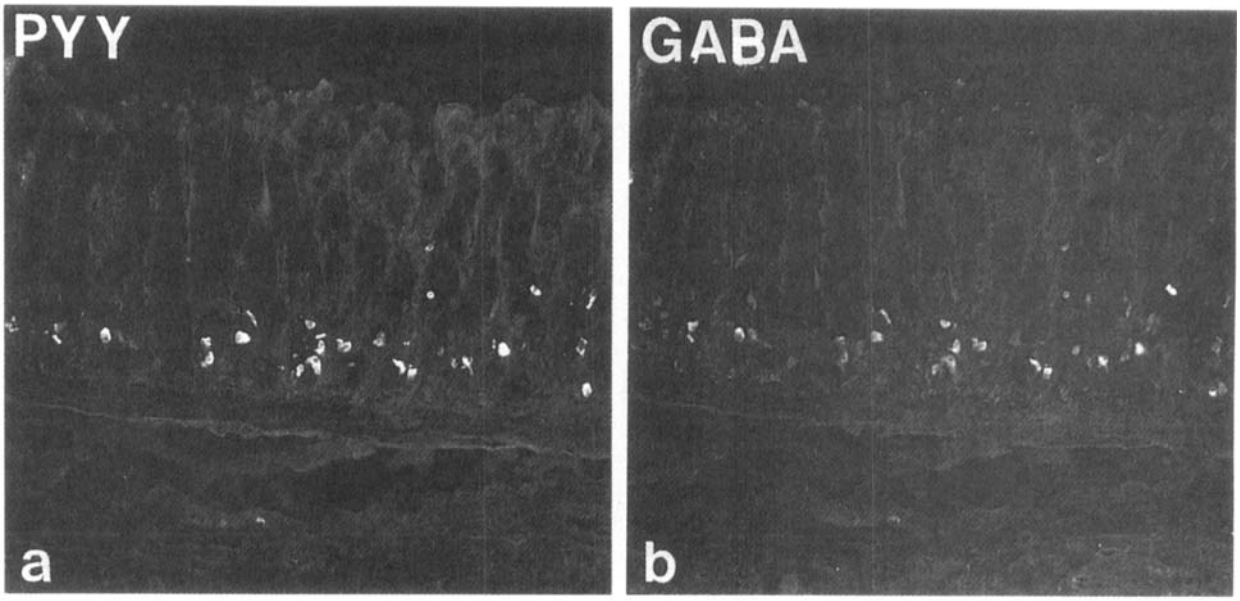


Fig. 5. Rat antral mucosa. Cryostat section processed for the simultaneous demonstration of a) PYY and b) GABA. Most of the PYY-immunofluorescent cells display also GABA immunofluorescence of varying intensity. (× 130).

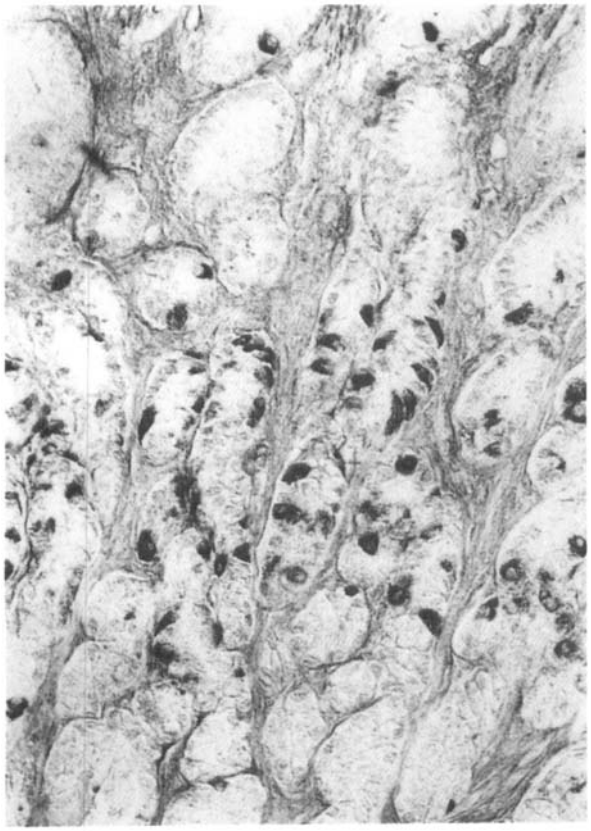


Fig. 6. Human antral mucosa. Section immunostained for xenopsin. The xenopsin-immunoreactive cells, which are numerous in the mid-portion of the glands are identical with gastrin cells (c.f. ref. 46). (× 250).

by proteolytic processing, has been demonstrated in many antral endocrine cells, including the gastrin cells (48). The functional significance of the coexistence of several candidate messengers is a matter of speculation. In contrast to gastrin, but like pancreastatin, both PYY and GABA inhibit acid secretion. Since all three putative messengers are present also in intestinal and islet endocrine cells it is reasonable to assume that they have paracrine or autocrine functions rather than endocrine.

Gut neuropeptides—late additions

During the last few years neuromedin U (NMU) and pituitary adenylate cyclase activating peptide (PACAP) have emerged as regulatory peptide candidates with a localization to neurons in the gut (Figs 7 and 8).

NMU was first isolated from porcine spinal cord extracts (49) and was found to stimulate uterine motor activity (U for uterus). NMU occurs in the pig in two different forms, NMU-8 and NMU-25, NMU-8 constituting the C-terminal octapeptide of NMU-25. In the rat NMU has 23 amino acids (NMU-23) and in this species there is no cleavage signal like the Arg-Arg sequence at positions 16 and 17 in the porcine NMU-25 (50, 51). Thus, NMU-23 is probably not processed to smaller fragments. NMU occurs in the gut predominantly in the intestines. In the mouse, rat, guinea pig and pig intestine NMU-containing fibers are quite numerous in the mucosa and submucosa and in the myenteric ganglia (52–56). NMU-containing fibers are thought to be intrinsic to the gut since nerve cell bodies containing the peptide are regularly seen in submucous and myenteric ganglia and since extrinsic denervation does not overtly affect the NMU fiber density in the gut wall as studied in the guinea pig (55) and rat (Ekblad et al., to be published).

In the guinea pig, NMU-immunoreactive neuronal elements are richly distributed in the small intestine. In the submucous ganglia NMU was found to coexist with either VIP, NPY or SP in three different subpopulations of nerve cell bodies. In addition, NMU-immunoreactive endocrine cells were found to occur in the intestinal epithelium (55). In the pig small intestine NMU-immunoreactive nerve cell bodies are numerous in submucous ganglia and here NMU was found to coexist with CGRP and substance P (56).

PACAP is a VIP-like peptide first isolated from ovine hypothalami and found to occur in two forms, PACAP-27, and a C-terminally extended form, PACAP-38 (57, 58). PACAP-27 displays 68% sequence homology with VIP. The ovine and human PACAP genes have been cloned and the predicted human PACAP sequence is identical with the ovine peptide (59). PACAP-immunoreactive neuronal elements have been demonstrated in the ovine hypothalamus (60). We have used a PACAP-27 antiserum that does not recognize VIP, PHI or secretin to examine the distribution of immunoreactive PACAP in the gut of rat, cat, pig and man by immunocytochemistry ((61) and Sundler et al., to be published). PACAP immunoreactivity was observed in nerve fibers in the gut wall of all species examined. In man PACAP-immunoreactive fibers were richly distributed in all layers of the gut whereas in the other species the fibers predominated in the myenteric ganglia and the muscle (Fig. 8). Delicate PACAP-immunoreactive fibers were, however, regularly seen in the gastric mucosa in all species. Immunoreactive nerve cell bodies were numerous in both submucous and myenteric ganglia of the human gut, while being relatively rare and restricted to the myenteric ganglia in the other species examined. Double immunostaining revealed coexistence of PACAP and VIP throughout the human gut and in the gastric mucosa of the other species examined. In the outer layers of the stomach and in the intestines of these latter species, the bulk of PACAP-immunoreactive nerve fibers were distinct from those storing VIP and also distinct from those storing substance P, enkephalin or somatostatin.

Very little is known about the functional role of NMU and PACAP in the gut. The results of studies of porcine intestinal mucosa in vitro suggest that NMU can modify intestinal ion transport via an interaction with non-cholinergic enteric neurons (62). Preliminary experiments

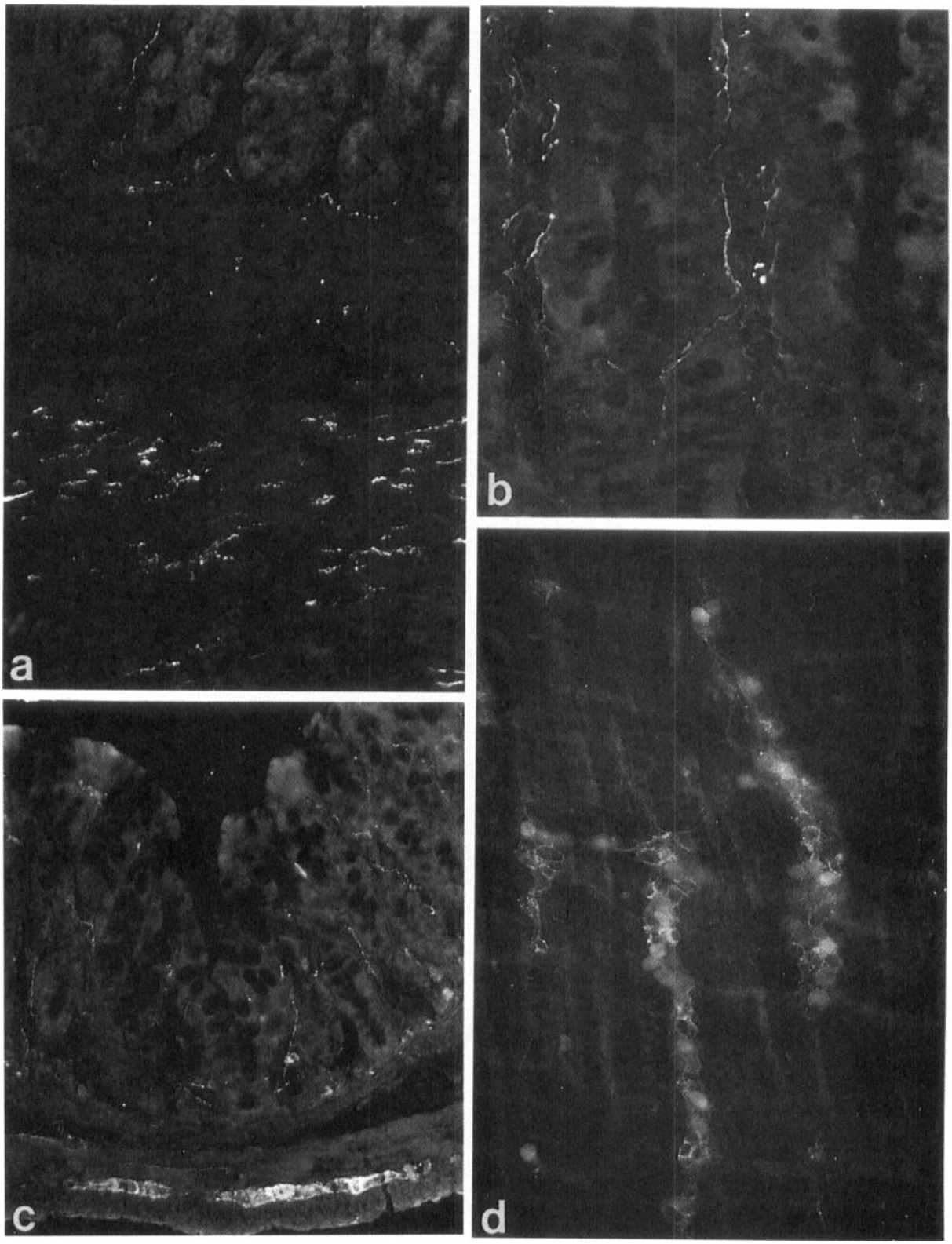


Fig. 7. Neuromedin U immunofluorescence in: a) chicken antrum, where immunoreactive nerve fibers are numerous in the smooth muscle, b) mucosa of rat small intestine where immunoreactive fibers run in the villus core, often close to the epithelium, c) mouse colon, where immunoreactive fibers are distributed in all layers with a predominance in the myenteric ganglia (bottom of fig.) and d) whole mount of longitudinal muscle with adherent myenteric ganglia. The myenteric ganglia are rich in neuromedin U-immunoreactive nerve cell bodies and nerve fibers (a; ×250, b; ×300, c; ×180, d; ×200).

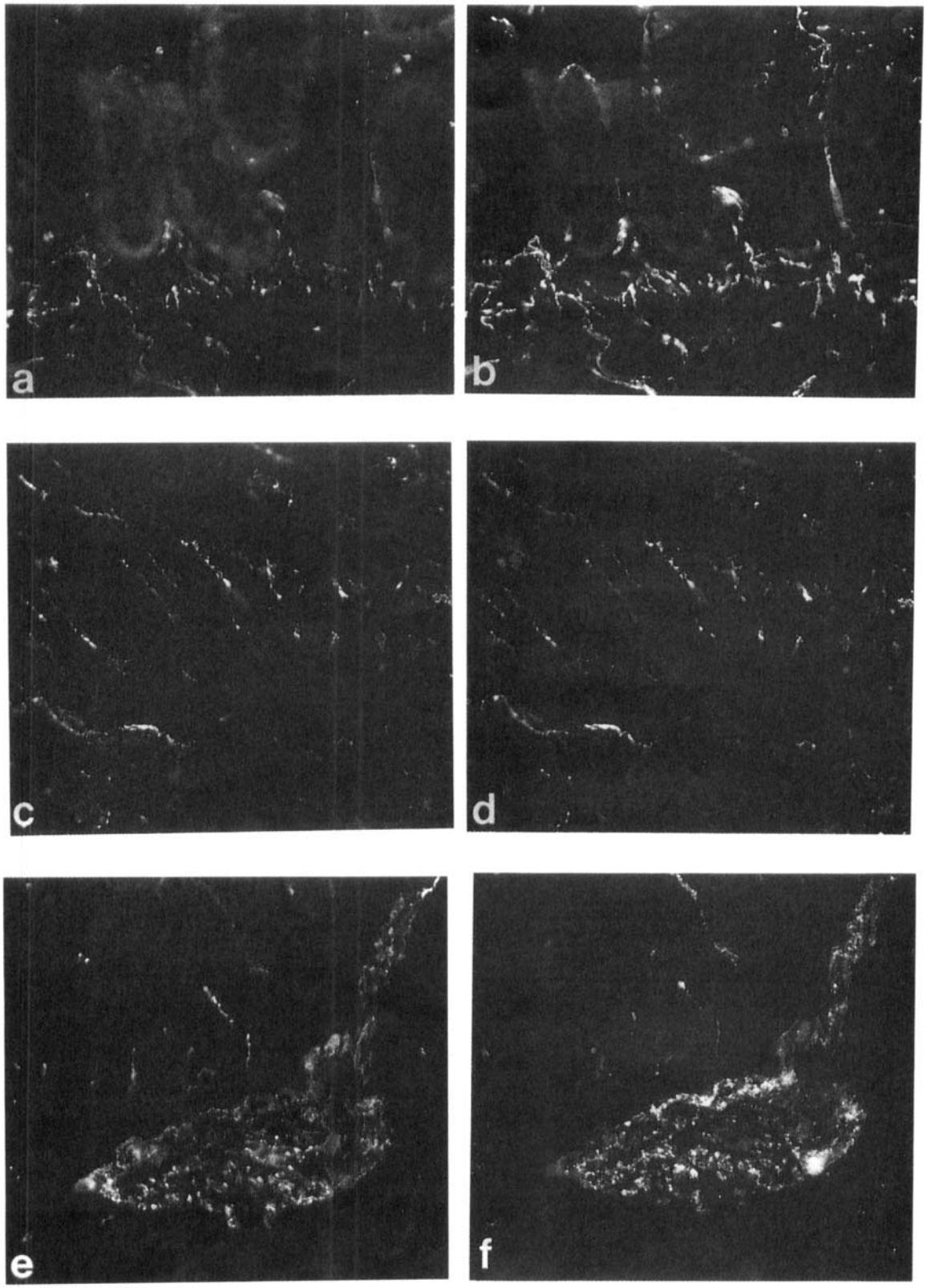


Fig. 8. Simultaneous double immunostaining for PACAP (a, c, e) and VIP (b, d, f). a and b = human colon, mucosa; c and d = human colon, circular muscle; e and f = cat antrum, smooth muscle with large myenteric ganglion. In human intestine PACAP-containing nerve fibers are identical with those storing VIP. In the gut of the cat (and many other animals) the vast majority of PACAP-containing nerve fibers are distinct from the VIP-containing ones (× 250).

on gut motility have revealed a contractile effect on turtle intestine of NMU-8 whereas there was no effect on the motility of the rat and guinea pig gut (63). Recently Maggi et al. (64) reported that NMU-8 contracts the longitudinal muscle of isolated human ileum. This effect was unaffected by tetrodotoxin, indicating a direct effect of NMU-8 on the smooth muscle cells. For PACAP, being a VIP-like peptide, it can so far only be speculated that it will exert VIP-like actions in the gut, such as smooth muscle relaxation, stimulation of intestinal fluid secretion and vasodilation. It may be mentioned in this context that PACAP and VIP show comparable vasodepressor activity (57). Further, the intestine is among the organs in the body that are richly supplied with PACAP binding sites (65).

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