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Somatostatin cells in rat antral mucosa: qualitative and quantitative ultrastructural analyses in different states of gastric acid secretion

R. Lamberts, D. Stumps, L. Plümpe, and W. Creutzfeldt

Department of Internal Medicine, Division of Gastroenterology and Endocrinology, Georg-August-University of Göttingen, Robert-Koch-Strasse 40, W-3400 Göttingen, Federal Republic of Germany

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Summary. In the gastrointestinal tract somatostatin is localized in endocrine cells and in neurons. The antral somatostatin (D-) cell shares features of both cell types. The activity of the antral D-cell is regulated by intragastric pH. Therefore different states of gastric acidity were induced experimentally in order to study D-cell morphology at the electron microscopical level. The morphological findings were related to measurements of plasma and tissue concentrations of the peptide. The D-cell is characterized by extensive membrane interdigitations with neighbouring cells. Changes in the activity of antral D-cells are reflected by an increase in cytoplasmic secretory granule density and a shift of secretory granules towards basal cell processes. Direct endocrine cell contacts at the level of the perikarya were rarely observed. The intracellular distribution of secretory granules suggests that cell communication is more likely to take place at the level of the strongly immunoreactive cytoplasmic processes. No evidence for endocrine or exocrine (luminar) secretion was observed morphologically. This is in agreement with the concept of paracrine secretion of the antral D-cell.

Introduction

Somatostatin is a tetradecapeptide that was originally isolated from hypothalamus by Brazeau et al. (1973) and has long been known to inhibit pituitary growth hormone secretion. A large number of immunocytochemical studies revealed somatostatin-like immunoreactivity (SLI) in a variety of different peripheral organs, including the pancreas, the gastrointestinal tract (Alumets et al. 1977, 1979; Lehy 1979), parafollicular C-cells of the thyroid, urinary tract, retina, placental tissue and submandibular gland (for review see Larsson 1985). In these tissues somatostatin is found both in typical endocrine cells as well as in neural structures (Costa et al. 1984). Due to the widespread localization of this peptide and its many different biological effects that are evoked by very small somatostatin concentrations, a local (paracrine) rather than a true endocrine way of action has been suggested (Creutzfeldt 1975, 1987; Saffouri et al. 1979) in accordance with the paraneuron concept originally proposed by Fujita (1976).

For the gastrointestinal tract this concept was further supported by the light microscopical observation of immunoreactive somatostatin cells with long basal processes often ending in club-like swellings on known somatostatin effector cells, i.e. gastrin cells in the antrum and parietal cells in the oxyntic mucosa (Helmstaedter et al. 1977; Larsson et al. 1979). However, direct electron microscopical evidence of endocrine cell contacts in the antrum is still missing (Canese 1977).

SLI is found both in veins draining the antrum as well as in the gastric lumen in response to various stimuli (Uvnäs-Wallensten et al. 1977). Therefore besides a paracrine way of action (Holst et al. 1983; Creutzfeldt 1987) endocrine release into blood vessels (Schusdziarra 1987) and exocrine secretion via the apical cell process in the "open type" antral D-cell has also been discussed but never been demonstrated at the electron microscopical level.

The regulation of antral somatostatin release is still incompletely understood (for review see Arimura et al. 1981; Schusdziarra 1983; McIntosh 1985; Lucey 1986). Somatostatin release is highly sensitive to luminal factors such as pH, i.e. the D-cell activity corresponds to intragastric acidity. Increased somatostatin release may inhibit the gastrin-producing G-cell (Bloom 1974; Creutzfeldt and Arnold 1978). This has been demonstrated both by infusion of somatostatin as well as by injection of anti-somatostatin γ-globulin in rats (Chiba et al. 1981; Short et al. 1985). An inverse correlation between antral gastrin and somatostatin cells has been demonstrated in humans under different states of gastric acid secretion: the G/D cell ratio was increased in states of reduced acid secretion (pernicious anaemia, vagotomy) and decreased in massive hyperchlorhydria (gastrinoma

Offprint requests to: R. Lamberts

patients), (Arnold et al. 1982). In order to study morphological aspects of somatostatin cell activity intraluminal pH is therefore the most important variable determining the grade of activity of the antral D-cell. In antral G-cells a secretory cell cycle, dependent on the activity of the cell, has been defined on the basis of morphological criteria at the EM level but has been controversially debated in the literature (Forssmann et al. 1969; Track et al. 1978; Hakanson et al. 1982). Therefore it was of interest to study quantitative and qualitative aspects of D-cell activity at the electron microscopic level. In addition the morphological data were related to measurements of plasma and tissue concentrations of the respective peptide.

Materials and methods

Male Wistar rats, weighing between 180 and 220 g, were kept at a 13-h day and 11-h night cycle (6 a.m. to 7 p.m.) and received food and water ad libitum.

The animals were divided into 4 groups and treated as follows:

a) Group 1 was given 1 ml of 0.1 *N* hydrochloric acid intragastrically every 15 min for 3 times via an intragastric tube. The animals were killed 15 min after the last HCl application ($n=12$).

b) In the second group a truncal vagotomy was performed and the animals were sacrificed on the third day after surgery ($n=15$).

c) In the third group the vagotomy was combined with a pyloroplasty and the animals killed 14 days after surgery ($n=26$).

d) The fourth group served as control ($n=16$).

All animals were fasted for 36 h before the experimental day. The experiments were done in the morning and all animals killed by guillotine between 10 a.m. and 12 a.m.. After opening of the thoracic and abdominal cavities blood was taken by heart puncture for the radioimmunological measurement of gastrin and somatostatin concentrations. The stomach was excised and antral tissue removed both for the morphological characterization of somatostatin cells and for the measurement of gastrin and somatostatin tissue concentrations.

Morphology

Tissue was fixed in Bouin's fluid for light microscopical immunocytochemistry on paraffin sections. For the electron microscopical investigations the tissue was fixed in Karnovsky's solution, postfixed with OsO_4 and embedded in Epon. Silver to gold ultrathin sections were cut on a LKB ultratome. For the immunocytochemical demonstration of somatostatin-like immunoreactivity an immunogold postembedding staining technique was applied which has been described in detail previously (Lamberts et al. 1990). The rabbit antisomatostatin antiserum was a gift from Dr. J. Polak, London, and was directed against somatostatin-14. It was used at a dilution between 1:16000 and 1:20000. The sections were contrasted with uranyl acetate and lead citrate and viewed in a Zeiss (Oberkochen, FRG) EM 9.

For the electron microscopical morphometry all sections were scanned carefully for immunocytochemically identified nucleated D-cells. Every cell was photographed at 2 fixed magnifications of 4700 and 7800 and the total cell reconstructed by photomontage. The cell was then divided into 3 parts: a) a nuclear and perinuclear region, defined as an area of 3 cm around the nucleus at a final magnification of ×23150; b) the apical part, located above the nucleus, often extending with a thin process to the glandular lumen; and c) a basal part, mainly representing cytoplasmic processes at the base of the gland, running for various distances along the basement membrane.

Using the Zeiss morphomate total cell area, nuclear area, number and size of secretory granules and the size of the Golgi apparatus were determined in each of the 3 cell regions.

Hormone extraction

For the measurement of tissue gastrin and somatostatin concentrations the peptides were extracted in boiling water according to a protocol described in detail elsewhere (Conlon et al. 1982). The results are expressed as pMol peptide/g wet tissue.

The values are shown as mean ± SEM. For the statistical evaluation a Student's *t*-test for unpaired values was employed and the significance level was set at $p<0.05$.

Results

Serum gastrin concentrations in fasted animals are shown in Fig. 1a. In comparison to control animals gastrin levels decrease significantly after intragastric hydrochloric acid application to about 50%. After vagotomy there is a prominent increase to about 10 to 14 times the control values, which is more pronounced immediately after surgery than 2 weeks later. In contrast only a minor increase in plasma somatostatin concentrations is observed in all 3 experimental groups in comparison to control animals (Fig. 1b), which is significant only in the long-term vagotomy group as well as after HCl-treatment.

The corresponding tissue concentrations of each peptide are shown in Table 1. The acute application of hydrochloric acid slightly increases the amount of gastrin extractable from the tissue which corresponds to the decreased gastrin release observed in serum. After short-term vagotomy (3 days) gastrin tissue concentrations are markedly decreased but are almost doubled by 2 weeks. Again changes in somatostatin tissue concentrations are less pronounced. Acute acid application does not significantly change tissue somatostatin, however, both short- and long-term vagotomy significantly decrease tissue concentrations to almost 60% of control levels.

The characteristic appearance of immunoreactive D-cells in rat antral mucosa at the EM level is shown in Fig. 2. The cells are found predominantly in the lower third of the gastric glands. They have a prominent nucleus, a well developed endoplasmic reticulum and Golgi apparatus and abundant secretory granules of low to medium electron density, homogeneous structure and a loosely fitting granular membrane. A conspicious feature of these cells are long, slender basal processes which always show an intense immunoreaction for somatostatin (Fig. 3). In many cases there is an elaborate interdigitation between the D-cell membrane and neighbouring cells, either at the level of the cell body or of the cytoplasmic processes as shown in Fig. 4a. Numerous prominent cytofilament bundles are found, predominantly around the nucleus, but are also seen extending into the cytoplasmic processes (Fig. 4b). In the apical part 1 or 2 centrioles were seen in several cases (Fig. 4b). The cell membranes show few membrane specializations like desmosomes (Fig. 4c). In the antrum many of the D-cells belong to the open type, sending a tender cytoplasmic process to the glandular lumen (Fig. 5).

The endocrine cells are mostly singular, however, in several cases a close juxtaposition of other endocrine (mostly gastrin or somatostatin) cells can be observed (Fig. 6). In these cases direct endocrine cell contacts are less frequent (Fig. 6a, b), but the cells are usually sepa-

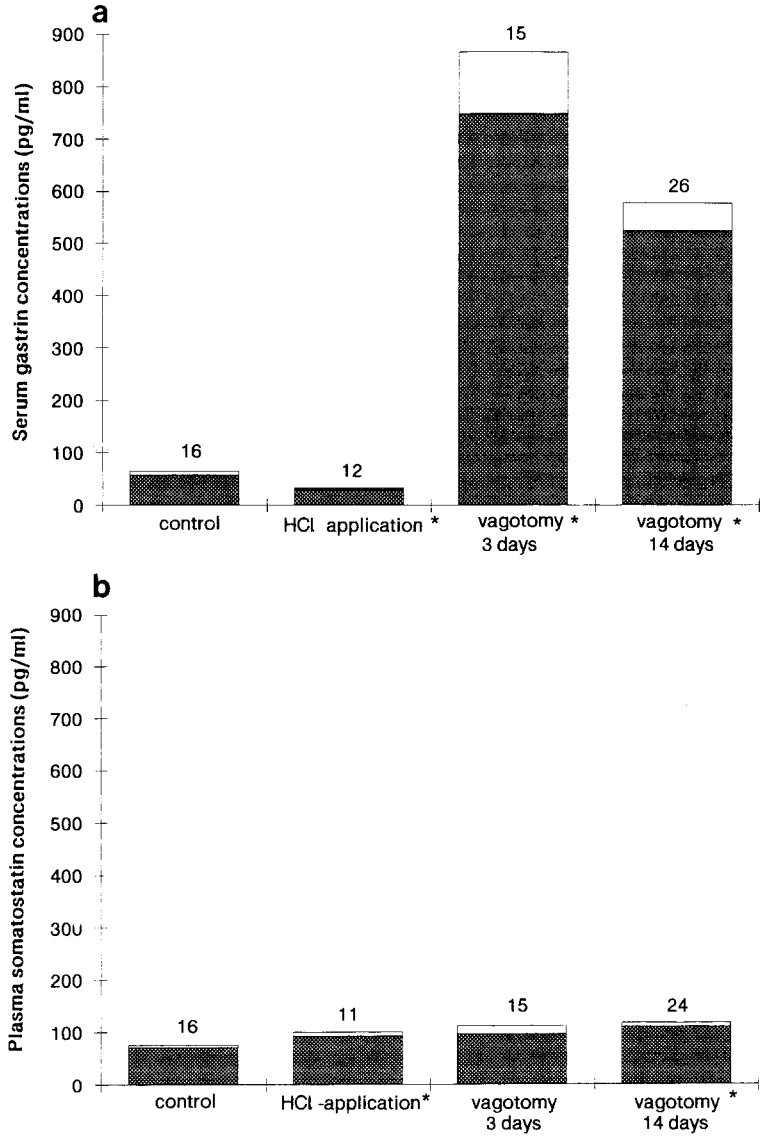


Fig. 1a and b. Serum gastrin (**a**) and plasma somatostatin (**b**) concentrations in the 4 experimental groups mean ■, SEM □; **a**, **b**: $*p<0.05$ vs. control group

Table 1. Gastrin and somatostatin tissue concentrations in experimental animal groups

	Control	HCl application	Vagotomy 3 days	Vagotomy 14 days
Gastrin concentration (nmol/g)	8.82	10.74	4.24*	8.34**
SEM	1.84	1.53	0.35	1.13
n	12	10	15	12
Somatostatin concentration (nmol/g)	0.36	0.35	0.25*	0.23*
SEM	0.03	0.03	0.02	0.02
n	12	12	15	26

$*p<0.05$ vs. control group; $**p<0.05$ vs. vagotomy 3 days

rated by thin cytoplasmic processes of non-endocrine (usually mucus-producing) cells (Fig. 7).

At the electron microscopical level a total of 149 nucleated D-cells (control:30, HCl application:35, vagotomy (3 days):53 and vagotomy (14 days):31) in 23 rats were measured. The exact number of cells with apical or basal processes in each of the 4 experimental groups is shown in Table 2. In the 4 groups the nuclear area (expressed as percentage of total cell area) did not change, the nucleus representing about 45 to 50% of the total cell area in single sections. The secretory granule density increased in HCl-treated rats in comparison to the control group (8.71 vs. 8.10%) and decreased in the two vagotomy groups (6.64 and 5.42% respective-

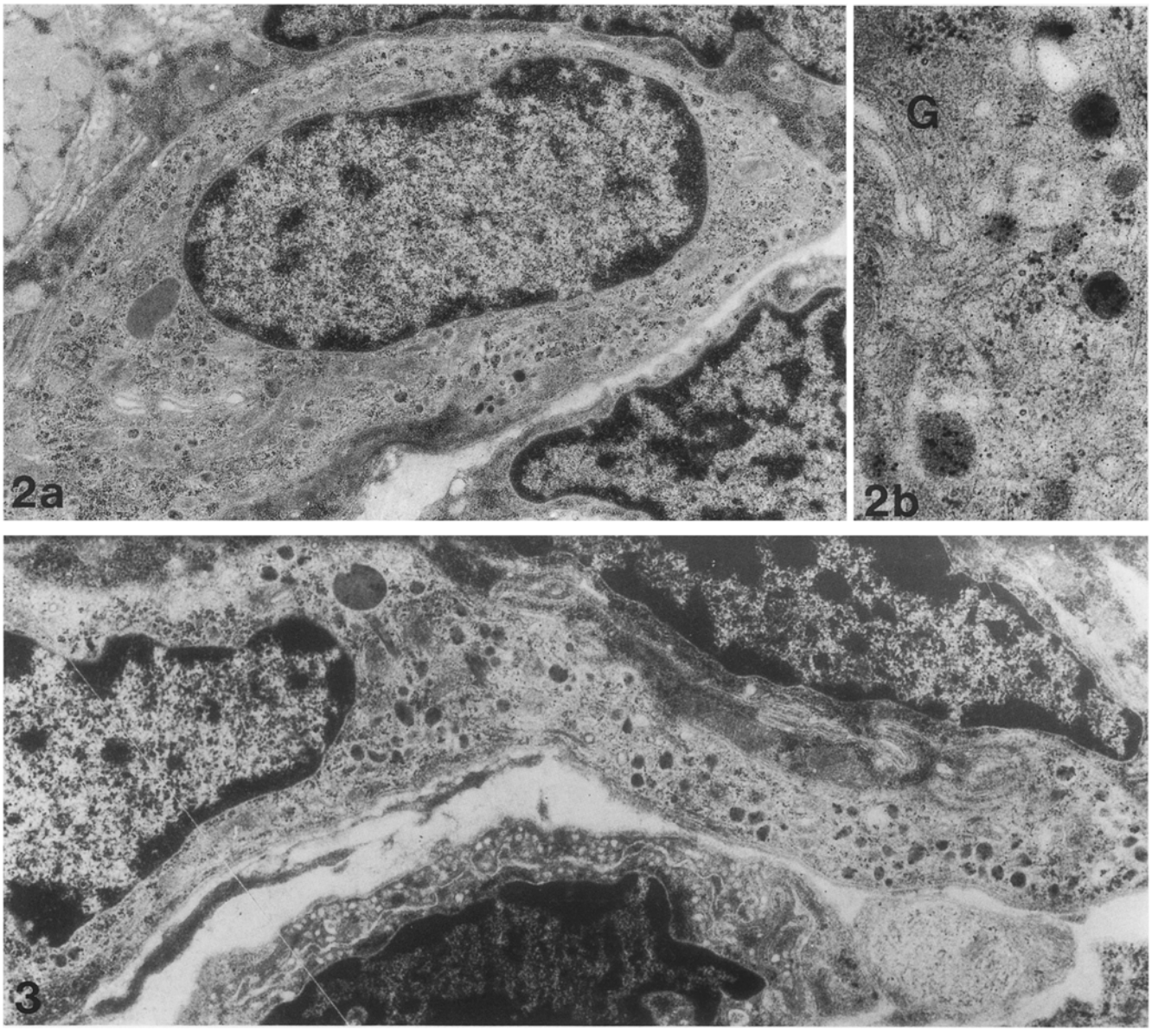


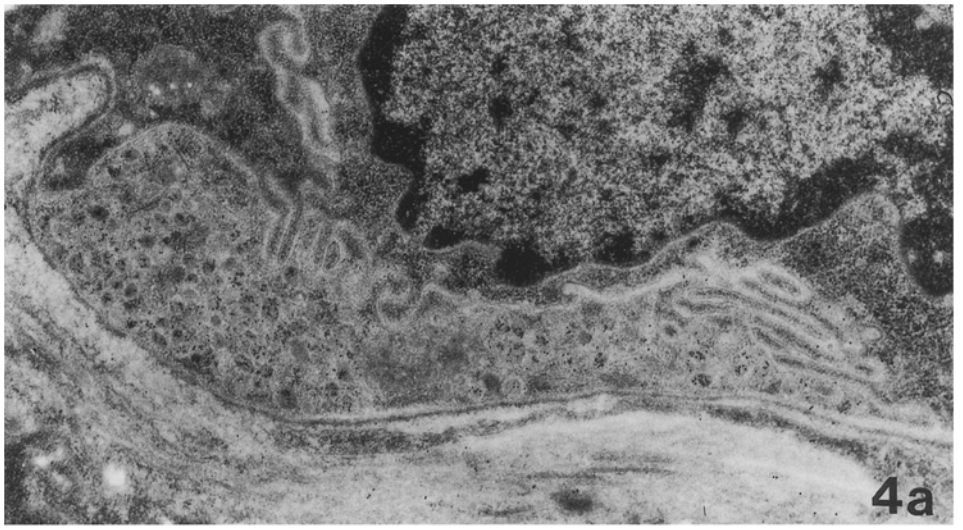
Fig. 2. a Immunoreactive D-cell in rat antral mucosa. Note the prominent nucleus, well developed ER and Golgi apparatus as well as the numerous secretory granules. (Immunogold postembedding staining, anti-somatostatin 1:16000, magnification ×12340). **b** At a higher magnification (×41785) the secretory granules have a low to medium electron density, a homogeneous structure and a loosely fitting granular membrane. The immunogold particles are restricted to mature secretory granules, no labeling besides background is found on ER and Golgi apparatus (*G*)

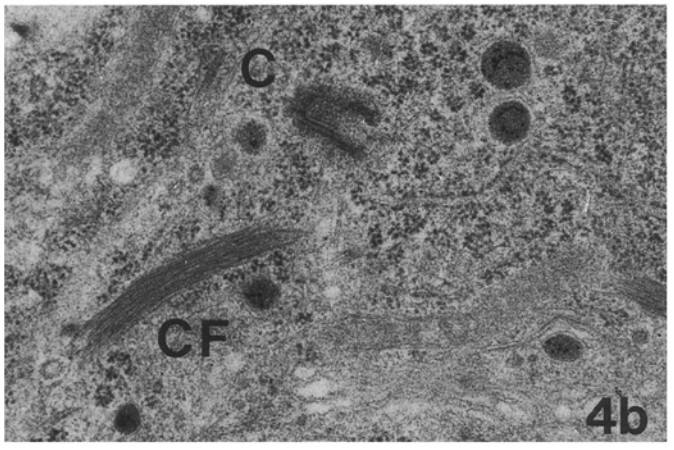
Fig. 3. D-cell process running for a long distance along the basement membrane. The process is filled with numerous strongly immunoreactive secretory granules. (Magnification ×13660)

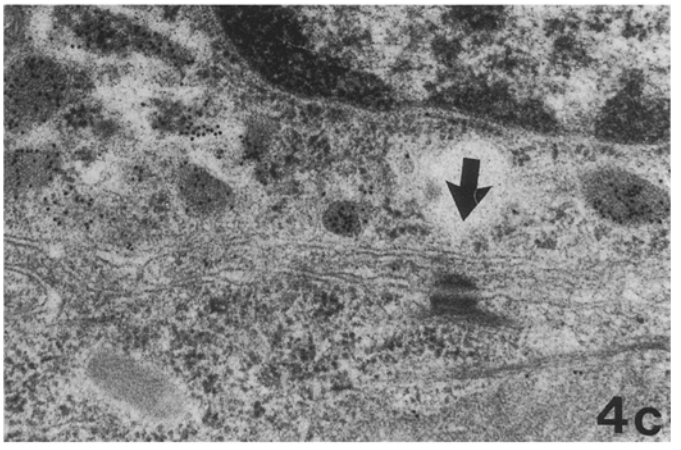
ly, Table 3), the decrease being significant by 14 days after vagotomy.

Looking at the 3 subcellular regions, the distribution of secretory granules was similar in all animal groups with a high density in the basal processes and a relatively lower density in the apical processes. In comparison to control rats after HCl treatment total secretory granule density increased mainly due to an increase in the perinuclear compartment (8.18 vs. 7.68%) with a concomitant decrease (4.46 vs. 5.5%) in the apical cell area (Table 3). The decrease of secretory granule density after vagotomy was mainly due to changes in the basal compartment (8.75 vs. 13.98%). These acute changes in secretory granule density in subcellular compartments were due to a shift of the granules from one compartment to another as indicated in Table 4: the relative distribution of secretory granules in the perinuclear region increased from 67 to 78.1% after HCl treatment and decreased in the apical part from 13.4 to 6.4%. No changes in the relative distribution of secretory granules were observed in long-term vagotomized animals.

The Golgi apparatus was found predominantly in the peri- and supranuclear region, only about 6% of the total Golgi area was located in the basal part of







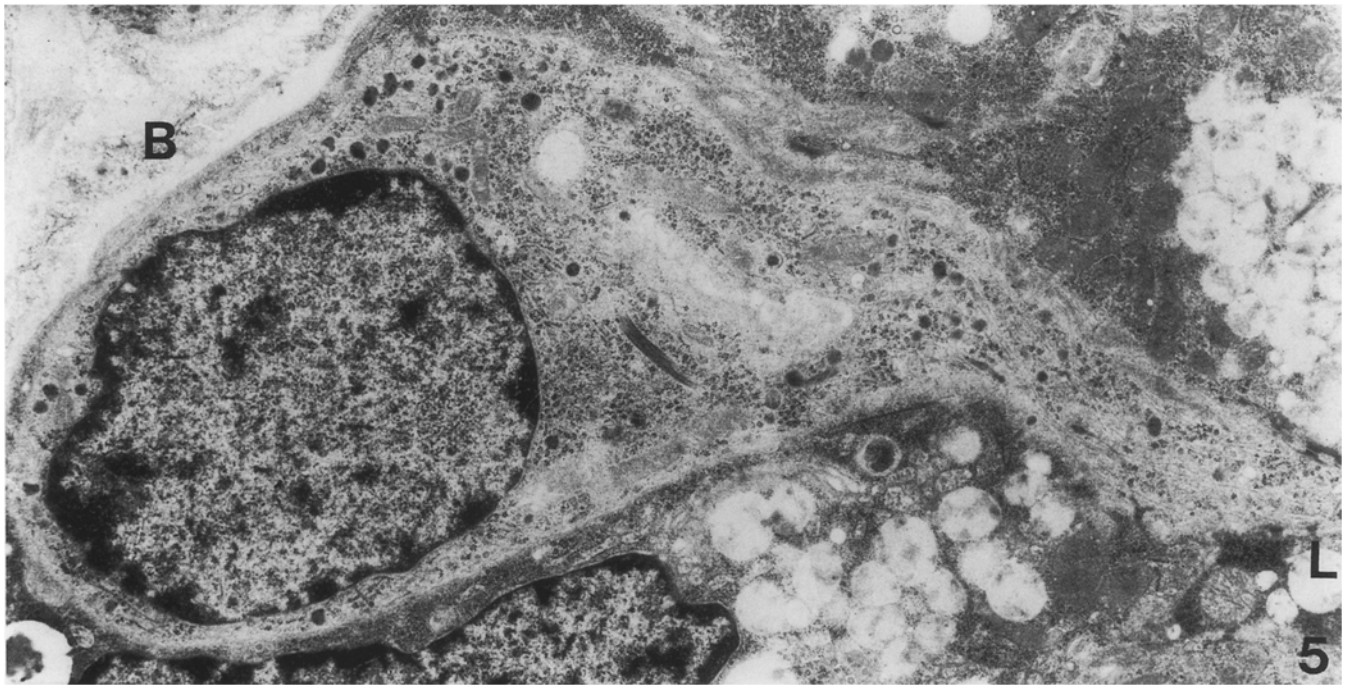


Fig. 4. Electron microscopic characteristics of antral D-cells: **a** Elaborate membrane interdigitations between the D-cell membrane and neighbouring cells at the level of a basal cytoplasmic process. (Magnification ×14670). **b** A prominent cytofilament (*CF*) bundle close to the nucleus, a centriole (*C*) in the apical part of the cytoplasm. (Magnification ×26590). **c** Membrane specializations between an antral D-cell and a neighbouring cell. (Magnification ×37360)

Fig. 5. "Open type" D-cell in rat antral mucosa. A thin cytoplasmic process extends from the base (*B*) to the glandular lumen (*L*). (Magnification ×10490)

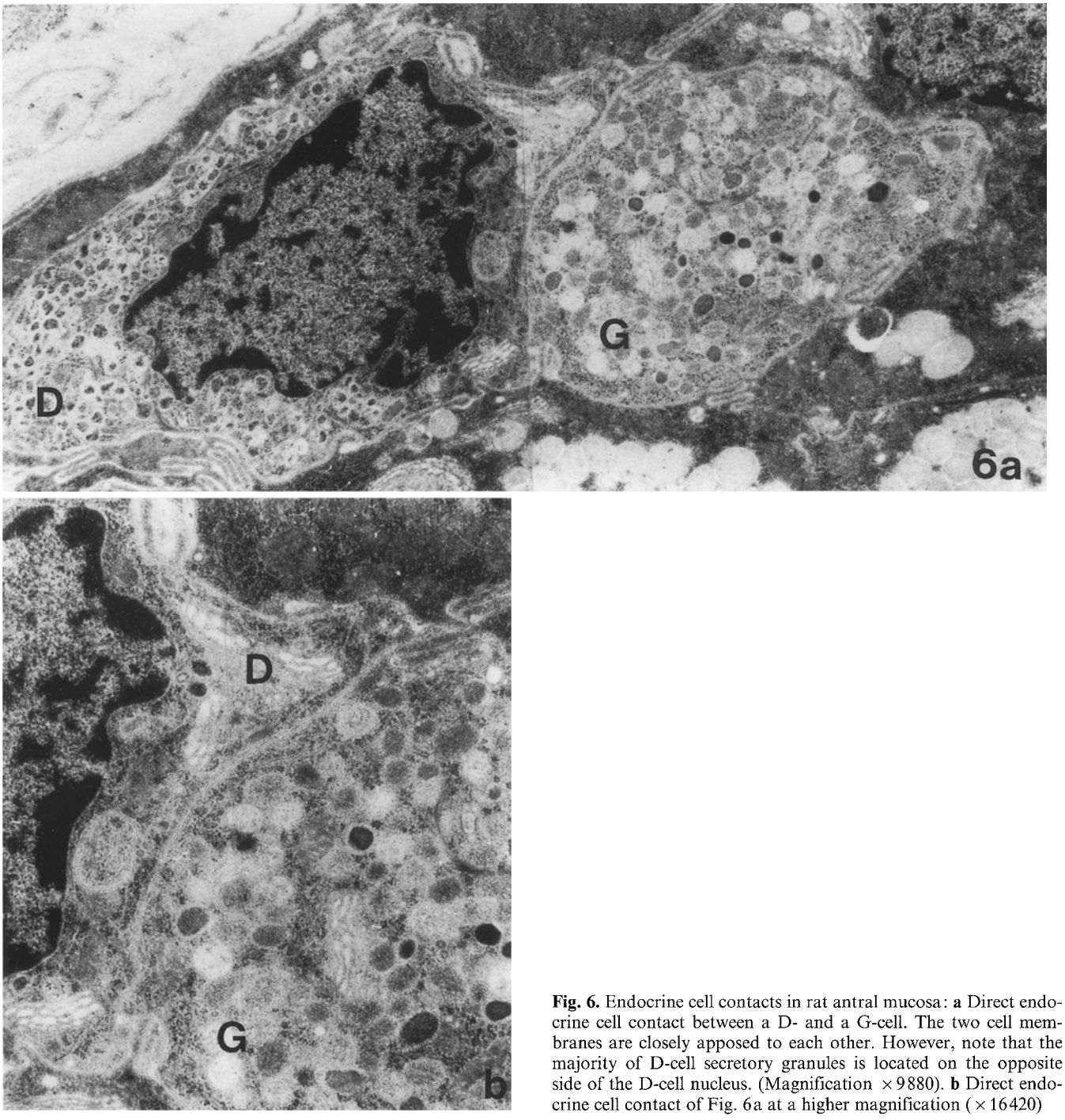


Fig. 6. Endocrine cell contacts in rat antral mucosa: **a** Direct endocrine cell contact between a D- and a G-cell. The two cell membranes are closely apposed to each other. However, note that the majority of D-cell secretory granules is located on the opposite side of the D-cell nucleus. (Magnification ×9880). **b** Direct endocrine cell contact of Fig. 6a at a higher magnification (×16420)

the cytoplasm. After vagotomy there was a shift towards basal areas (increase from 6 to about 11%), the opposite trend was observed after HCl-application (Table 4).

Discussion

In the present study the effects of changes in intragastric acidity on the electron microscopical characteristics of the antral D-cell were examined and related to tissue and plasma concentrations of the respective hormone. There was a positive correlation between secretory granule density and D-cell activity, combined with a shift of secretory granules from apical towards perinuclear and basal compartments after acute D-cell stimulation by HCl.

In the gastrointestinal tract the major molecular form of somatostatin in mucosal endocrine cells (with the exception of the gastric antrum) is somatostatin-28, while intramural neurons, pancreas and antrum contain mainly the tetradecapeptide (Penman et al. 1983). The antiserum used in this study was a polyclonal rabbit antiserum

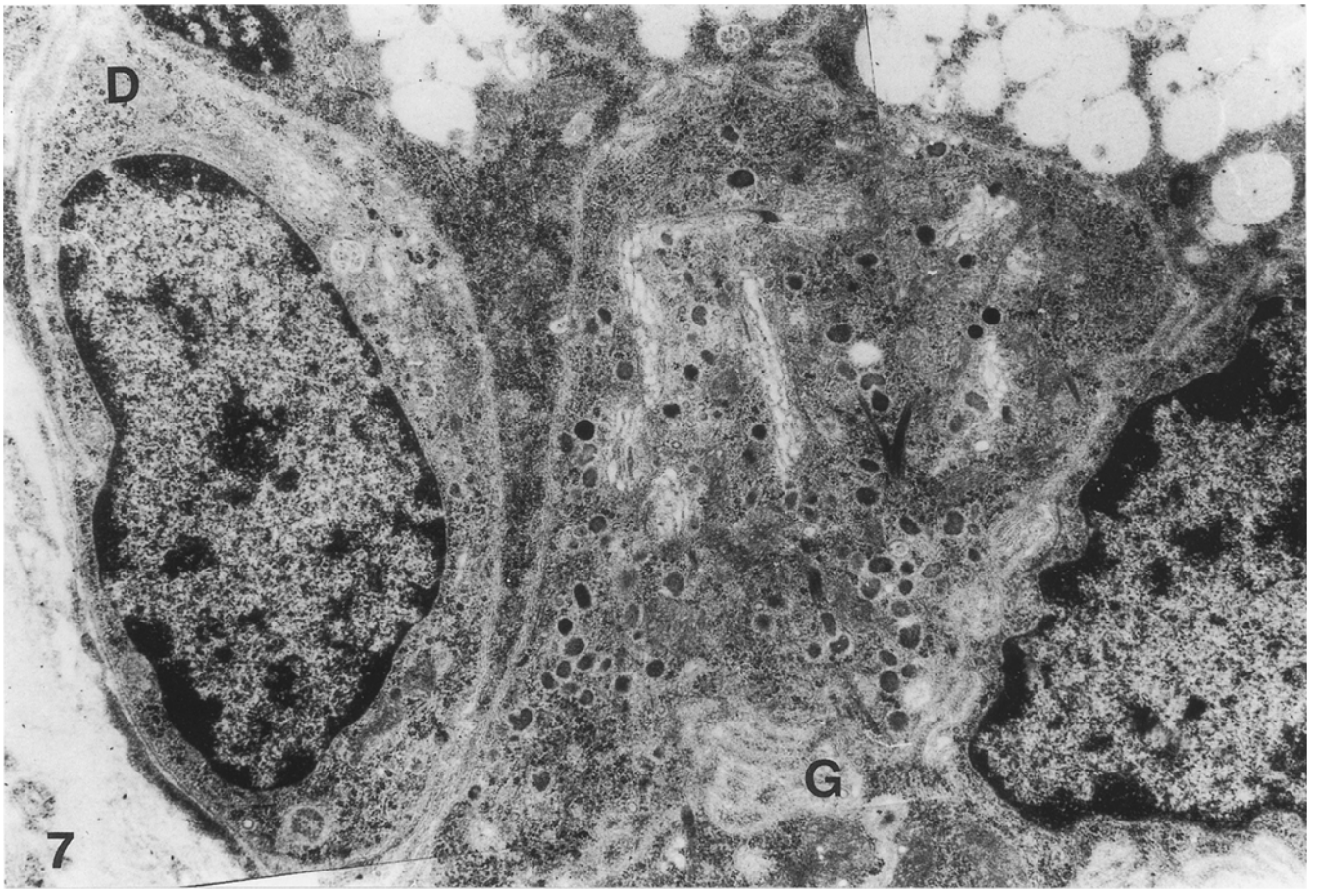


Fig. 7. Indirect D-G-cell contact. The perikaryal membranes are separated by a thin intervening process of a non-endocrine cell. (Magnification ×12290)

Table 2. Number of D-cells with apical and/or basal processes

	Control		HCl application		Vagotomy 3 days		Vagotomy 14 days	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Total number of cells	30	100	35	100	53	100	31	100
Apical processes	9	30	11	31	10	19	13	42
Basal processes	12	40	16	46	25	47	11	35

Table 3. Morphometric analysis of antral D-cells at the electron microscopical level

	Mean total cell area (μm^2)	Nuclear area (%)	Perinuclear area (%)	Apical processes (%)	Basal processes (%)	SG density/ total cytoplasmic area (%)	Apical SG density (%)	Perinuclear SG density (%)	Basal SG density (%)	Golgi area/ cytoplasmic area (%)
Control	21951.00	44.97	38.22	32.50	14.45	8.10	5.50	7.68	13.98	2.88
HCl application	20387.00	48.19	41.12	17.24	12.53	8.71	4.46	8.18	11.60	3.38
Vagotomy 3 days	18457.00	45.45	39.95	24.73	17.80	6.64	4.68	5.92	10.27	4.08
Vagotomy 14 days	22625.00	47.30	37.80	19.10	16.62	5.42*	3.92	5.19	8.75	3.33

*$p < 0.05$ vs. control group

Table 4. Relative distribution (%) of Golgi apparatus and secretory granules (SG) in subcellular compartments in different experimental groups

	Control	HCl application	Vagotomy 3 days	Vagotomy 14 days
Golgi area (%)				
Apical	27.2	41.3	10.8	2.5
Perinuclear	66.9	58.7	78.6	86.5
Basal	5.9	0.0	10.6	10.9
SG area (%)				
Apical	13.4	6.4	8.1	12.1
Perinuclear	67.0	78.1	65.2	69.0
Basal	19.6	15.5	26.7	18.9

directed against somatostatin-14, no exact quantitative data on the crossreactivity with somatostatin-28 are available. However, it gave comparably good staining results in both antral and oxyntic mucosa.

Gastric somatostatin plays an important role in the regulation of gastric acid secretion. Oxyntic somatostatin cells may inhibit parietal cells directly, antral D-cells reduce gastric acid secretion indirectly by inhibition of antral G-cell activity, possibly via an interaction at the nuclear level (Brand et al. 1988). Many of the observed actions of somatostatin in the gastrointestinal tract are pH dependent. This further supports the role of acidity as the main determinant when studying D-cell function. The validity of the experimental model used in this study is reflected by the tissue concentrations of the native peptide: assuming predominantly a paracrine secretion of somatostatin acute stimulation of D-cell activity by hydrochloric acid instillation is unlikely to be paralleled by changes in plasma or tissue concentrations. However, in the resting state the concentration of the peptide is reduced, dependent on the "turnover" of mature secretory granules and cell replication time. The latter has been estimated to be around 40–60 days in the rat (Lehy 1982). Opposite changes were seen in gastrin tissue concentrations: a drop in intragastric pH was followed by an increase in the amount of gastrin extractable from the tissue, corresponding to a decreased G-cell activity. On the contrary, no "steady state" was reached 3 days after vagotomy when tissue levels had dropped in comparison to controls in order to maintain extremely high serum concentrations.

EM characteristics of the antral D-cell

The ubiquitous localization of somatostatin throughout the body as well as its many diverse functions favour the idea of a local (paracrine) rather than an endocrine way of action. The concept of paracrine secretion is supported by the light microscopical immunocytochemical demonstration of long basal processes of antral somatostatin cells which were often seen in close contact to other endocrine, namely gastrin cells (Larsson et al. 1979). The present electron microscopical results are in good agreement with the light microscopical data, demonstrating several long basal cytoplasmic D-cell processes densely packed with secretory granules. The immunoreactivity is almost exclusively localized over these secretory granules, due to the intracellular concentration gradient of the peptide from the endoplasmic reticulum to the Golgi apparatus, the sensitivity of the immunocytochemical method and the specificity of the antiserum. In about 30% of the cells an apical cell process, extending from the supranuclear area to the gastric lumen was found, classifying the antral D-cell as an open cell type. However, this number is probably underestimated due to the limited section thickness and the orientation of cutting. The fact that the percentage of basal processes identified was reduced (40 vs. 35%) and that of apical processes increased (30 vs. 42%) after long-term vagotomy might indicate a certain plasticity in the number and size of the processes as also suggested by Kusumoto and Grube (1987). Prominent cytofilament bundles as well as centrioles are a characteristic feature of many endocrine cells in the gastrointestinal tract, their exact function in these cells is not known. Cytofilament bundles might facilitate cell process "plasticity" or the oriented movement of secretory granules within the cell.

Secretory granule "plasticity"

In all experimental animal groups secretory granule density was highest in the basal processes, in accordance to the light microscopical observation of accumulation of strongly immunoreactive material at the basal cell pole. In vagotomized rats secretory granule density decreased concomitantly in all 3 subcellular compartments, reflecting the decreased D-cell activity. After hydrochloric acid application into the stomach there was an increase of secretory granule density combined with a shift of granules towards perinuclear and/or basal cell compartments. Anterograde transport of secretory granules in D-cell processes has been demonstrated by Larsson (1984) who employed a double labeling immunofluorescent technique to separately label old and new secretory granule fractions in different fluorescent colours. No evidence was found in this study for an apical granule release. Granules found in the apical cytoplasm might have pinched off the Golgi apparatus that was often located in the supranuclear region. Somatostatin immunoreactivity in the gastric lumen (Uvnäs-Wallensten 1977) would then be an "overspill" phenomenon from the intercellular (basolateral) space unless a subpopulation of antral D-cell serves a true exocrine function as well. Whether the prominent cytofilament bundles are involved in a basally directed transport of secretory granules within the D-cell is at present not known. Further studies employing various antibodies specific for somatostatin precursor molecules (Ravazzola et al. 1983) might be helpful in the demonstration of secretory granule maturation and transport within the antral D-cell.

Endocrine cell contacts

Direct endocrine cell contacts at the level of the cell body were rarely observed in this study. In cases where a close apposition of a second endocrine cell body was noticed these two perikarya were usually separated by a thin intervening process of a non-endocrine cell. This observation is not necessarily in contradiction to the above mentioned light microscopical results: the intervening processes are far beyond the resolution of light microscopy and serial sectioning through possible areas of contact was not done in the present study. However, due to the orientation of the secretory granules within the cell the results do not favour the idea of an endocrine interaction at the level of the cell body but rather support a communication via the cytoplasmic processes.

Granule release

The location of granule release has long been controversially debated in the literature. There are mainly two possibilities: release could take place all along the process in a paracrine fashion or it could be restricted to the terminal part of the process that is often seen as a club-like swelling at the light microscopical level. Data in support of the first concept come from Fujita et al. (1971) who observed exocytotic (omega) figures in antral endocrine (presumably D-) cells after HCl-stimulation. On the contrary, using serial sections Kusumoto et Grube (1987) claimed all D-cell processes to extend to capillaries, thereby strongly supporting the concept of endocrine secretion, probably through a local circulatory system. The design of our study does not allow to distinguish between these two possibilities because the ending of a cytoplasmic process cannot be defined by electron microscopy on single thin sections. However, direct contacts with capillaries were not observed which would strongly support a paracrine rather than an endocrine release.

In conclusion the light microscopical characterization of the antral somatostatin cell is further validated at the electron microscopical level. Changes in the activity of antral D-cells are reflected by an increase in secretory granule density and a shift of granules towards the perinuclear area and basal cytoplasmic processes. The data propose a polar orientation of antral D-cells within the mucosa with an apical receptor and a basal effector pole. No exact conclusion can be drawn with regard to the mode of secretion of the gastric D-cell: electron microscopical evidence for exocrine and endocrine secretion is scarce, the data fit best with the concept of paracrine secretion. A direct endocrine cell communication at the level of the cell perikarya seems unlikely in view of the intracellular distribution of secretory granules but rather takes place at the level of the processes that markedly increase cell surface (and contact) area of the antral D-cells.

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