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Immunocytochemical and autoradiographic studies of the endocrine cells interacting with GABA in the rat stomach

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Summary. There are now increasing evidences suggesting that GABA is able of direct interaction with certain endocrine cells. In the present study, highly specific anti-GABA-glutaraldehyde antibodies and ^{3}H-GABA uptake were used at the light and electron microscope levels to investigate the occurrence of cells containing endogenous GABA or taking up exogenous GABA in the mucosal antrum and corpus of the rat stomach. Only certain endocrine cell types of both regions were immunostained or grain-labelled. However, the morphology of their secretory granules did not allow to identify the nature of their hormone with certainty but suggested that somatostatin-like cells could interact with GABA. The combination of gastrin and somatostatin immunodetection with ^{3}H-GABA uptake autoradiography at the light microscope level, revealed that a subpopulation of somatostatin-like cells and other still unidentified endocrine cells are able to take up GABA, while the gastrin-like cells are not. These results reinforce the hypothesis that certain endocrine cell types of the diffuse endocrine system of the digestive tract are able to directly interact with GABA.

Introduction

Gamma-aminobutyric acid (GABA) is not only a major inhibitory neurotransmitter in the central nervous system of vertebrates, but also a peripheral neuromodulator, as in the enteric nervous system (Jessen et al. 1987). A few years ago, the presence of GABA was thought to be restricted only to nerve cells. However, recent studies have revealed its presence and importance in various non-neural organs such as the ovary, adenohypophysis, liver, digestive tract and pancreas. In these organs, several categories of endocrine cells feature as well morphological as physiological indications of direct interaction with GABA, i.e. prolactin- (Erdö 1985; Anderson and Mitchell 1986; McCann and Rettori 1986; Melis et al. 1986), insulin-, and somatostatin-secreting cells (Okada 1986; Garry et al. 1987a; Gilon et al. 1987a, 1988; Gilon and Remacle 1989). Moreover, certain of them have been reported to contain GABA: the insulin-secreting cells of the pancreatic islets possess as well the γ-aminoacid as the enzymes of its synthesis (GAD) and degradation (GABA-T) (Vincent et al. 1983; Okada 1986; Wu et al. 1986; Garry et al. 1987b); endocrine cells of the rat duodenum were also found to be immunoreactive to anti-GABA antibodies (Gilon et al. 1988).

In the stomach, another entity of the diffuse endocrine system, the secretion of certain hormones has already been reported to be regulated by GABAergic mechanisms (Harty and Franklin 1983, 1986; Harty and Murthy 1986). Nevertheless, morphological demonstration of direct interaction between GABA and endocrine cells was still lacking. Very recently, GABA immunoreactivity and ^{3}H-GABA uptake were shown in gastric epithelial cells, the nature of which remaining unknown (Jessen et al. 1988; Davanger et al. 1989).

The present paper reports high-resolution autoradiographic and immunocytochemical experiments aiming at localizing high-affinity GABA uptake and GABA immunoreactivity in antrum and corpus of the rat stomach. In addition, the combination of immunocytochemistry of hormonal peptides with the localization of ^{3}H-GABA uptake allowed to partially determine the nature of the endocrine cells which are involved or not in this uptake.

Materials and methods

Immunocytochemical detection of GABA

GABA antiserum. The raising and specificity studies of the antiserum have been described elsewhere (Seguela et al. 1984). Briefly, the immunogen, GABA-glutaraldehyde-carrier protein complex, was repeatedly injected into rabbits, and the titre of the antisera was checked by successive radioimmunological tests for 4 months. Its specificity was assayed in equilibrium dialysis by competition between the ligand and related amino-acids conjugated in the same way as the immunogen.

Immunocytochemical procedure. Wistar rats (1–3 months of age) were anaesthetized with diethylether, and perfused through the aorta with 50 ml washing solution (NaCl 9‰, procaïne hydrochloride 1‰ and glucose 1‰ in water) followed by 150 ml of 5% glutaraldehyde in 0.1 *M* phosphate buffer pH 7.2 (PB). Selected areas of the brain (cerebral cortex, striatum, and hippocampus), antrum and corpus regions of the stomach were dissected and postfixed in the same fixative solution for 1 h at 4° C. After two rinses for

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20 min in 0.05 *M Tris*-buffered saline solution (TBS) pH 7.4, 50 µm thick sections were cut on a Vibratome (Bio-Rad, Richmond, USA) before pre-embedding immunodetection. The free-floating sections were incubated for 20 min in 1% H_2O_2 to remove endogenous peroxidase activity, washed in TBS, incubated for 20 min in 0.2% Triton X-100 in TBS, washed again and preincubated in TBS containing 20% newborn calf serum (NBCS). Immunocytochemical visualization of GABA was then carried out as follows: overnight at 4° C in rabbit GABA antiserum diluted 1:5000 in TBS containing 1% NBCS, 2 washes for 20 min in TBS, 20 min in TBS containing 20% NBCS, 1 h at room temperature in anti-rabbit IgG antibodies (Bio-Yeda, Rehovot, Israel) diluted 1:100 in TBS and 1% NBCS, 2 washes for 20 min in TBS, 20 min in TBS containing 20% NBCS and 1 h at room temperature in rabbit peroxidase anti-peroxidase complex (Bio-Yeda, Rehovot, Israel) diluted 1:100 in TBS containing 1% NBCS. After extensive washings in TBS and then 0.05 *M Tris*-buffer (TB), the peroxidase activity was revealed by immersing the sections in TB containing 0.05% diaminobenzidine (Sigma, Saint-Louis, USA) and 0.01% H_2O_2 for 20 min. Control experiments were performed on tissue sections by omitting the primary antibody and by preincubating it in the presence of the reduced conjugates GABA- or β-alanine-glutaraldehyde.

After rinsing in PB, the sections were postixed for 1 h in 2% osmium tetroxide in PB, dehydrated through graded series of ethanol concentrations and embedded in Epon 812. Before polymerisation, the Vibratome sections were observed and photographed with a Zeiss Photomicroscope III. Selective areas of polymerized sections were cut off and glued on Epon supports for further sectioning on a Reichert Ultracut microtome. Semithin (1 µm thick) sections were stained with toluidine blue and photographed with the light microscope. Ultrathin (100 nm) sections were observed in a Jeol 100 C electron microscope without counterstaining.

Incubation procedure in the presence of ^{3}H-GABA

Wistar rats aged 2.5, 7.5 and 75 days post natum were used. Pieces (about 1 mm^3 each) of antrum and corpus regions of the stomach were dissected immediately after sacrifice. They were incubated for 30 min at 37° C in 2% albuminated Krebs solution (pH 7.2) containing 0.5 µ*M* ^{3}H-GABA (4-amino-n-(2,3-^{3}H)butyric acid, specific activity 78–105 Ci/mmol, Amersham, England), 1 m*M* AOAA (amino-oxyacetic acid, Janssen Chemica, Beerse, Belgium) and 1 m*M* β-alanine which allows to partially control the specificity of the uptake (Gilon et al. 1987a, b).

Single autoradiographic detection of the GABA uptake

After incubation in the presence of ^{3}H-GABA, the tissues were fixed for 1 h in 2.5% glutaraldehyde in PB, washed in PB, postfixed for 1 h in 1% osmium tetroxide in PB and dehydrated through a graded series of ethanol concentrations before epon embedding. Semithin and ultrathin sections were cut as before. They were processed for low- and high-resolution autoradiography as described elsewhere (Gilon et al. 1987a, b).

Coupling of immunodetection of hormonal peptides with autoradiographic detection of GABA uptake

Fixatives and tissue preparation. After incubation in the presence of ^{3}H-GABA, the tissues were immersed in several fixatives to compare the quality of the resulting immunocytochemical and autoradiographic stainings. Among the fixatives used were: (1) glutaraldehyde 0.1%, 0.2%, 0.3% or 2% in 0.1 *M* PB pH 7.4 for 1 h followed by overnight immersion in Bouin's fluid; (2) glutaraldehyde 0.1%, 0.2%, 0.3% or 2% in Bouin's fluid for 1 h followed by overnight immersion in Bouin's fluid; (3) glutaraldehyde 0.1%, 0.2%, 0.3% or 2% + paraformaldehyde 4% in 0.1 *M* PB pH 7.4 for 1 h followed by overnight immersion in paraformaldehyde 4% in 0.1 *M* sodium bicarbonate buffer pH 10.4 according to Eldred et al. (1983); (4) overnight immersion in Bouin's fluid alone; (5) immersion for 1 h in 2.5% glutaraldehyde in 0.1 *M* PB pH 7.4.

After fixation, the pieces were washed, dehydrated and embedded in paraffin. Sections (6 µm thick) were cut using a Reichert Biocut 2030 microtome and placed on chrome-alum gelatin coated slides.

Immunocytochemical detection of hormonal peptides. In order to restore the antigenicity of the tissue fixed with glutaraldehyde, deparaffinized sections were incubated for 30 min in 1% $NaBH_4$ (Eldred et al. 1983). They were then washed in TBS 0.05 *M* pH 7.4, incubated for 20 min in 1% H_2O_2, washed again and immersed in TBS containing 0.1 *M* monochloride lysine and 1% NBCS. After 20 min preincubation in 10% NBCS in TBS, they were incubated for 2 days at 4° C in the presence of rabbit somatostatin (Sera-Lab, Crawley Down, UK), cholecystokinin (CCK-39, CRB, Cambridge, UK) and gastrin G-34 (LW21) (Rahier et al. 1987) antisera all diluted 1:1000 in TBS containing 1% NBCS. After washing twice for 20 min in TBS, the sections were immersed in 10% NBCS in TBS and the primary antibodies were detected as previously described for the immunodetection of GABA.

Specificity controls included omission of primary antibody and preabsorption of the homologous antigen (somatostatin 14, gastrin G-17 I and 1–17 gastrin G-34 for the somatostatin, CCK-39 and gastrin G-34 antisera, respectively; all from Bioproducts, UCB, Braine-l'Alleud, Belgium).

Autoradiography. After extensive washing in TB and double distilled water, some slides were dried and covered with a 1% celloïdin film to control that the autoradiographic labelling did not result from a chemographic reaction (Gilon and Remacle 1989). The slides were then dipped in Ilford L4 emulsion (diluted 1:3 in water) and exposed for 3–5 weeks at 4° C. After developing, the sections were stained with hemalum of Mayer and photographed as before.

Results

Immunocytochemical detection of GABA

In control experiments performed on brain and stomach sections, no immunopositive labelling could be detected when the primary antibody was omitted or preincubated in the presence of the reduced conjugate GABA-glutaraldehyde. By contrast, preincubation of the anti-GABA antibodies with a hundredfold excess of the reduced conjugate β-alanine-glutaraldehyde did not affect the labelling (Fig. 1).

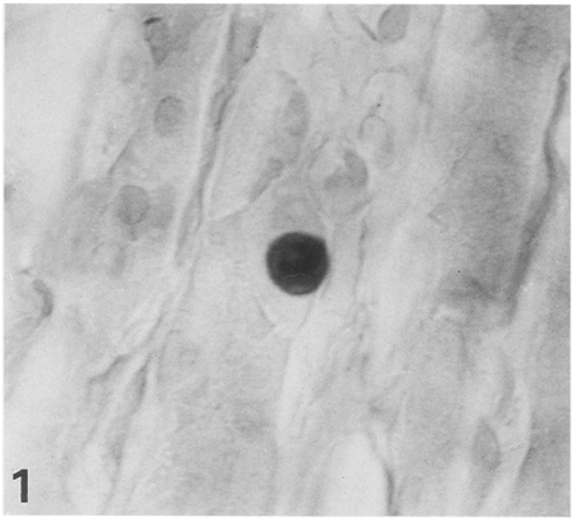
Fig. 1. Vibratome section in the stomach showing GABA-like immunoreactivity in an epithelial cell (presumably endocrine) after preincubation of anti-GABA antibodies in the presence of the reduced conjugate β-alanine-glutaraldehyde. × 730

Figs. 2–3. Semithin sections showing GABA-immunoreactivity in cells (*arrows*) inserted at the basal part of the epithelium (*E*); one of these cells (*arrowhead*) extends a tapering cytoplasmic process towards the lamina propria (*LP*). **2** × 740; **3** × 800

Fig. 4. Electron micrograph showing the GABA-immunoreactivity located in an endocrine cell (*En*) of the oxyntic epithelium. *BV* blood vessel; *LP* lamina propria; *Ox* oxyntic cell. × 6500

Fig. 5. Electron micrograph of a section through a gastric gland. An endocrine (*En1*) is immunostained whereas the other (*En2*), probably an ECL cell, is devoid of osmiophilic immunodeposit. *Ch* chief cell; *LP* lamina propria; *Ox* oxyntic cell. × 6300

Fig. 6. Electron micrograph of an immunoreactive endocrine cell containing round-shaped granules with a small halo under the membrane. *g* endocrine granule; *m* mitochondria. × 14000



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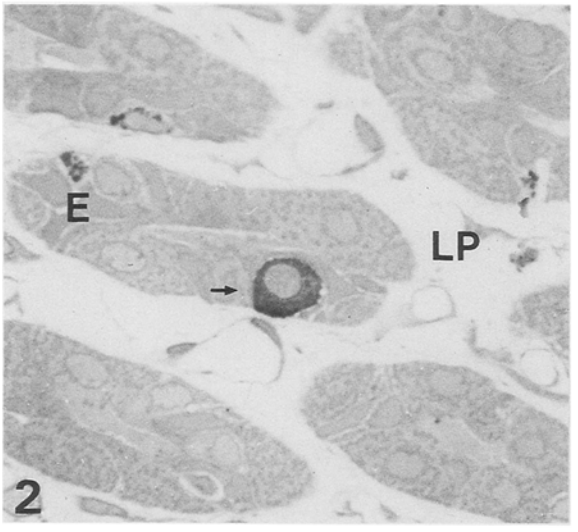

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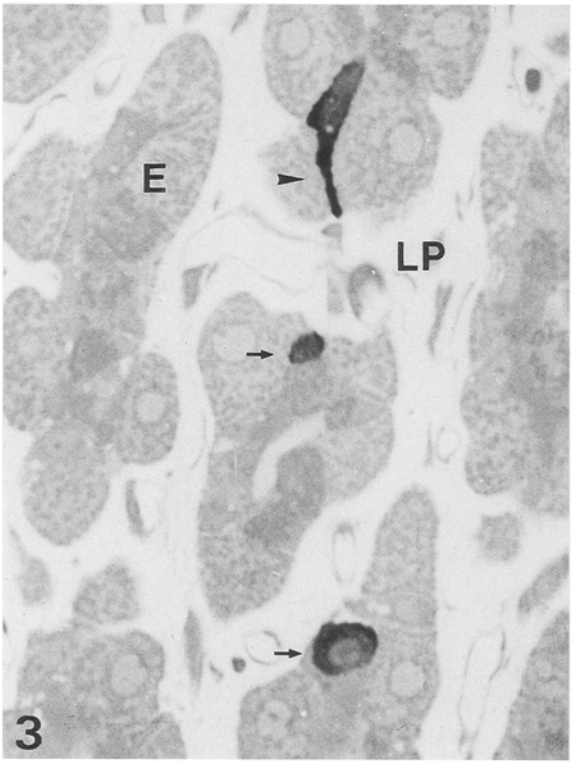

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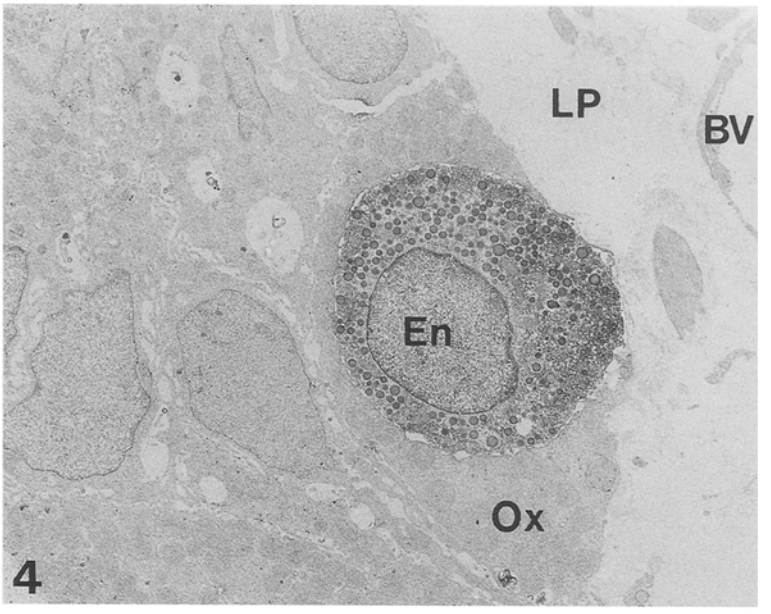

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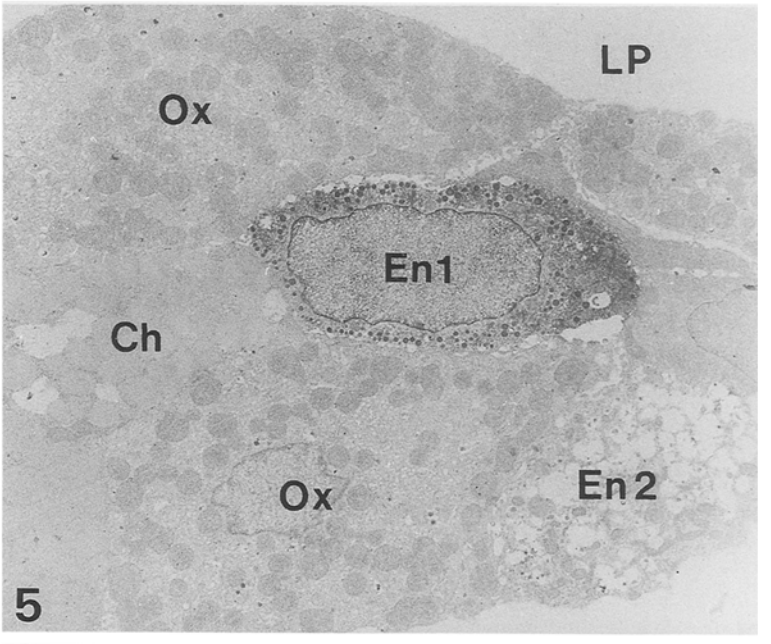

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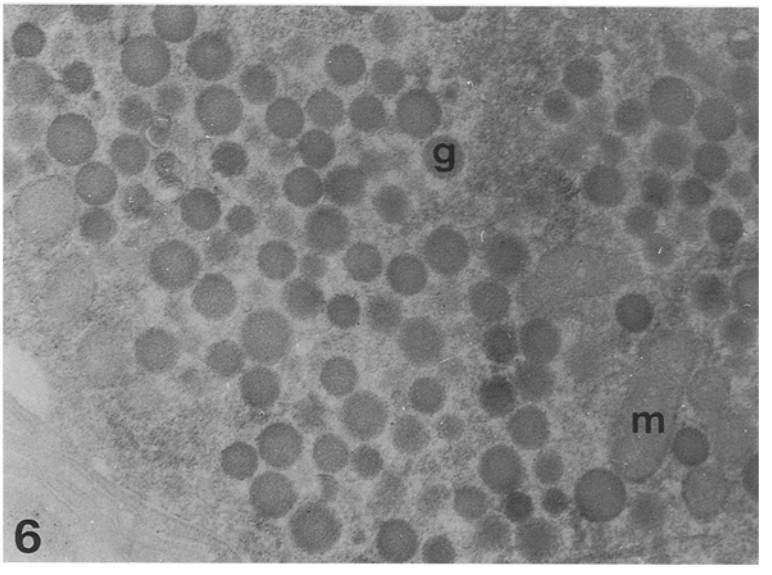

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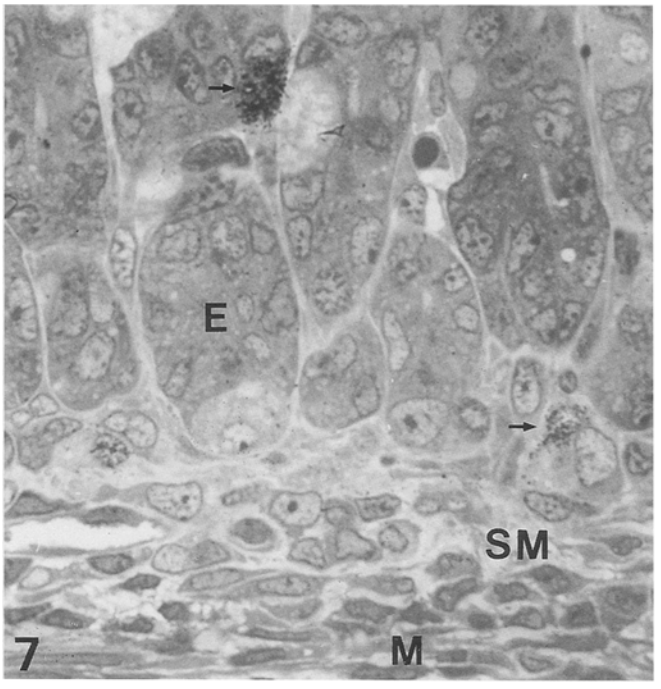
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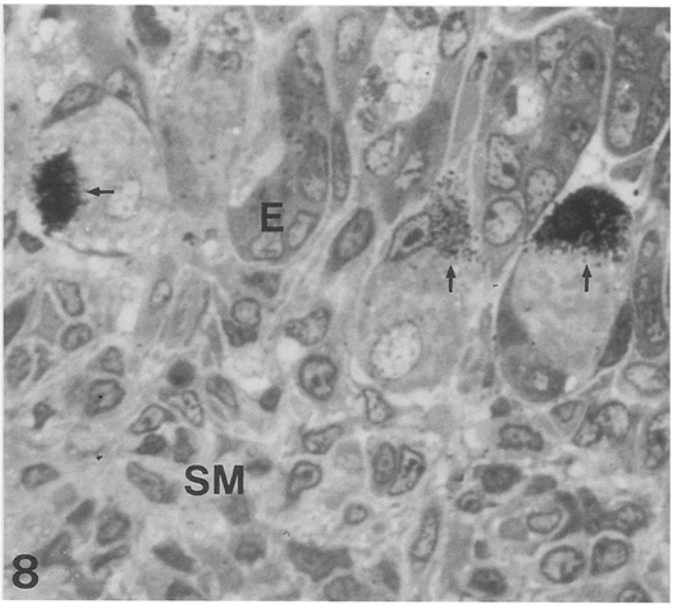
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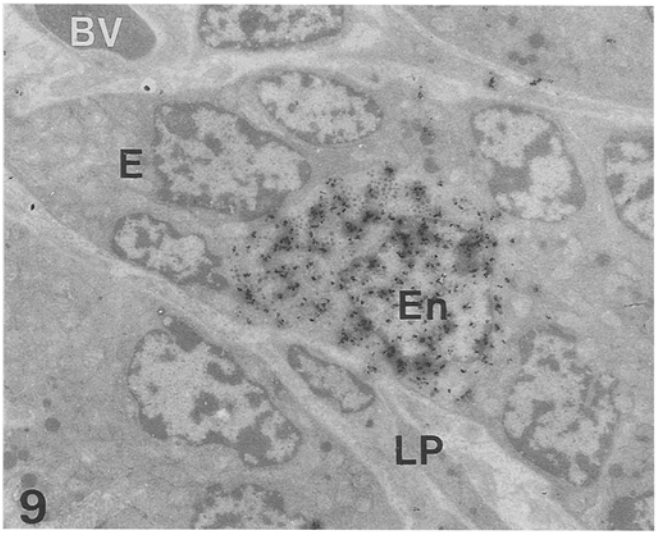
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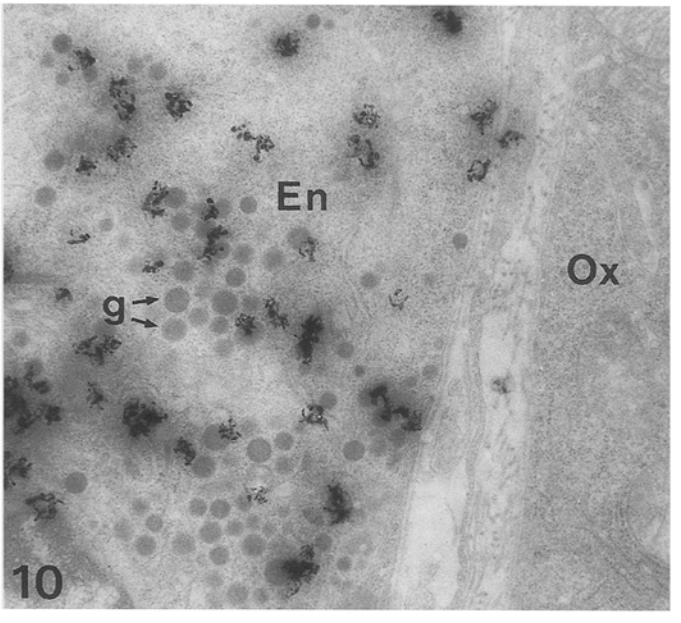
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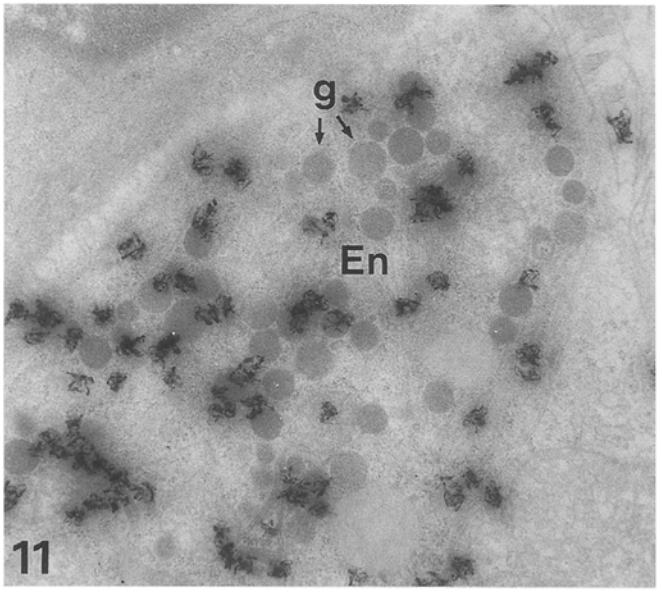
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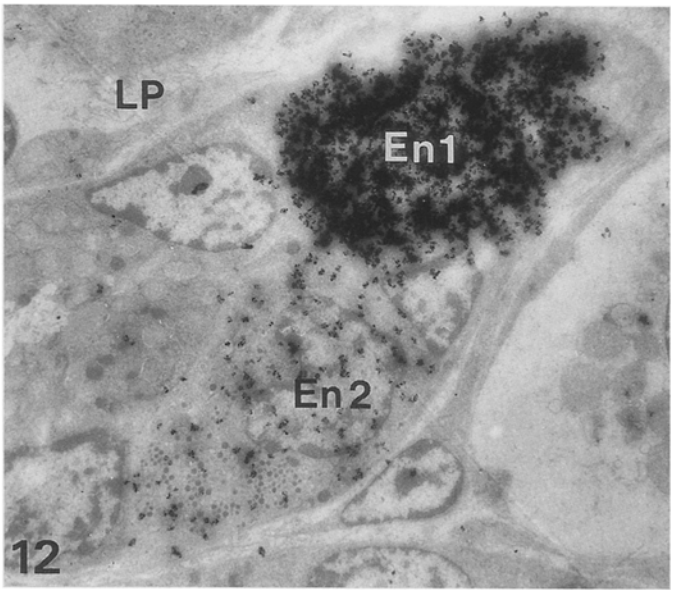
LP
En1
En2
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Stomach Vibratome sections incubated in the presence of the GABA antiserum showed an immunoreactivity in dispersed cells mostly located in the middle and lower parts of the gastric glands. They were uniformly distributed in the antrum and corpus. The cells were often round-shaped but some of them presented a tapering cytoplasmic process (Figs. 2, 3). The cytoplasmic labelling of the immunoreactive cells was always very intense whereas the labelling of the nuclei varied from cell to cell. Electron microscopy allowed to identify the immunoreactive cells as endocrine cells, owing to the presence of their secretory granules (Figs. 4, 5). The immunodeposit seemed to be restricted to endocrine cells featuring a similar morphology of the granules: the maximal diameter of these round granules reached 350–400 nm, and the core of low electron density was surrounded by a small halo under the membrane (Fig. 6). Nevertheless, these characteristics did not allow to assess the nature of the hormone.

Autoradiographic detection of GABA

Stomach fragments incubated in the presence of ^{3}H-GABA displayed a precise autoradiographic labelling on dispersed cells, the location of which in the middle and lower part of the glands (Figs. 7, 8), as well as the ultrastructural characteristics (Figs. 9–11), were similar to those described for GABA-immunoreactive cells. However, at least two different populations of labelled cells could be discerned on basis of the maximal diameters of the granules, that reached 380 and 250 nm respectively (Figs. 10, 11). The uptake ability of certain of them was very high as seen by the extremely intense labelling even after short time exposure (Fig. 12). In every case, the silver grains were dispersed uniformly as well on the nucleus as on the cytoplasm (Figs. 9, 12).

Immunodetection of hormonal peptides coupled with autoradiographic detection of GABA uptake

Effect of fixation. The effects of different procedures of fixation were tested on the immunoreactivity of gastrin and somatostatin as well as on the autoradiographic labelling (Table 1). Prefixation of the tissue with glutaraldehyde followed by postfixation in Bouin's fluid produced a high background for gastrin immunodetection and immunolabelling decreasing with higher glutaraldehyde concentrations. The use of paraformaldehyde gave negative results. By contrast, the combination of glutaraldehyde with Bouin's fluid in the prefixative produced immunolabelling as good as that obtained with the Bouin's fluid alone. For the autoradiographic labelling, the most intense signal was obtained in the presence of 2% glutaraldehyde in the prefixative. Consequently, 2% glutaraldehyde in Bouin's fluid has been found to be the mixture producing the best results for both techniques. It has been used in all the furter experiments.

Table 1. Effect of different procedures of fixation on the immunodetection of gastrin and somatostatin, and on the autoradiographic labelling in the rat stomach

Procedures of fixation	Immunoreactivity of		Autoradiographic labelling
	gastrin	somatostatin	
Glutaraldehyde in 0.1 *M* PB pH 7.4 for 1 h followed by overnight immersion in Bouin's fluid:			
Gluta 0.1%	+	++	0
Gluta 0.2%	BG	++	0
Gluta 0.3%	BG	+	+
Gluta 2%	BG	0	++
Glutaraldehyde in Bouin's fluid for 1 h followed by overnight immersion in Bouin's fluid:			
Gluta 0.1%	++	+	0
Gluta 0.2%	++	+	0
Gluta 0.3%	++	+	+
Gluta 2%	++	+	++
Glutaraldehyde + paraformaldehyde 4% in 0.1 *M* PB pH 7.4 for 1 h followed by overnight immersion in paraformaldehyde 4% in 0.1 *M* sodium bicarbonate buffer pH 10.4:			
Gluta 0.1%	0	0	0
Gluta 0.2%	0	0	0
Gluta 0.3%	0	0	+
Gluta 2%	BG	0	++
Overnight immersion in Bouin's fluid	++	++	0
Immersion for 1 h in 2.5% glutaraldehyde in 0.1 *M* PB pH 7.4	BG	BG	++

0: no labelling; +: weak labelling; ++: intense labelling; BG: intense background

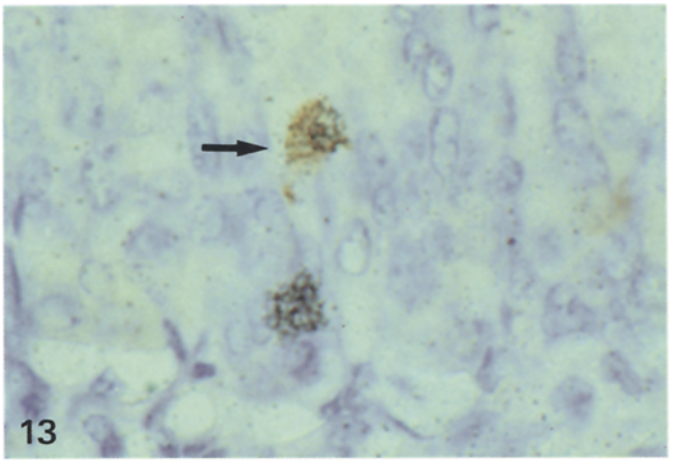
Analysis of the coupled techniques. Sections treated with the coupled techniques revealed three types of labelled cells in the stomach epithelium: (1) a majority of cells which were only autoradiographically labelled (Figs. 13–19); (2) cells which were only immunoreactive, it was the case of all the gastrin (Figs. 19–20), all the CCK-39 (Figs. 17–18) and the majority of the somatostatin-like cells (Figs. 14–16); (3) and cells which were both immunoreactive and grain labelled, it was the case of a small subpopulation of somato-

Figs. 7–8. Light micrographs of semithin sections of 7.5-day-old rat stomach fragments, incubated in the presence of ^{3}H-GABA, showing autoradiographic labelling in epithelial cells (*arrows*) of the gastric glands. *E* epithelium; *SM* submucous layer; *M* muscularis mucosae. **7** ×670; **8** ×740

Fig. 9. Electron micrograph of stomach epithelium of a 7.5-day-old rat showing silver grains over one endocrine cell (*En*) which has incorporated ^{3}H-GABA. *BV* blood vessel; *E* epithelium; *LP* lamina propria. ×2550

Figs. 10–11. Electron micrographs of two labelled endocrine cells (*En*) of 7.5-day-old rats. The secretory granules (*g*) of both cells are round-shaped with a small halo beneath the membrane but they are smaller in the cell seen on Fig. 10 (maximal diameter of 250 nm) than in the cell seen on Fig. 11 (maximal diameter of 380 nm). *Ox* oxyntic cell. **10–11** ×12000

Fig. 12. Electron micrograph of a 7.5-day-old rat oxyntic epithelium. The uptake of ^{3}H-GABA is extremely intense in an endocrine cell (*En1*) whereas it is weak in the other (*En2*). *LP* lamina propria. ×3800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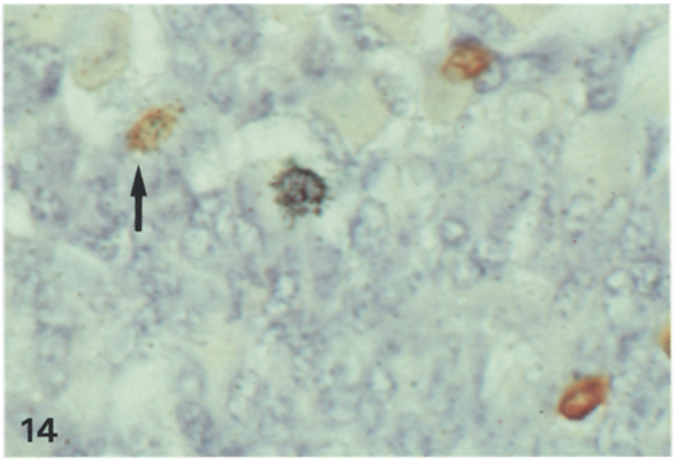
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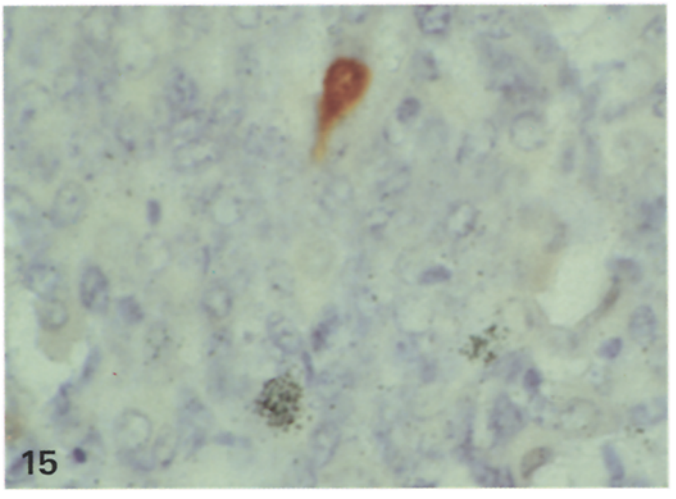
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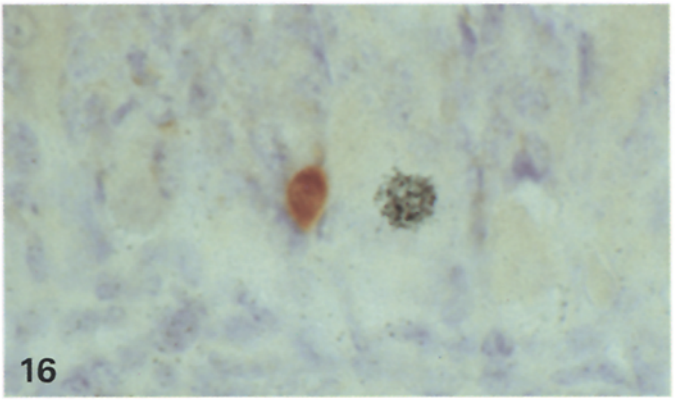
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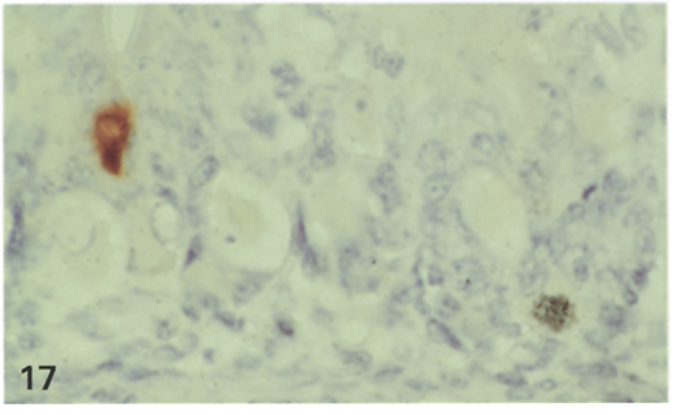
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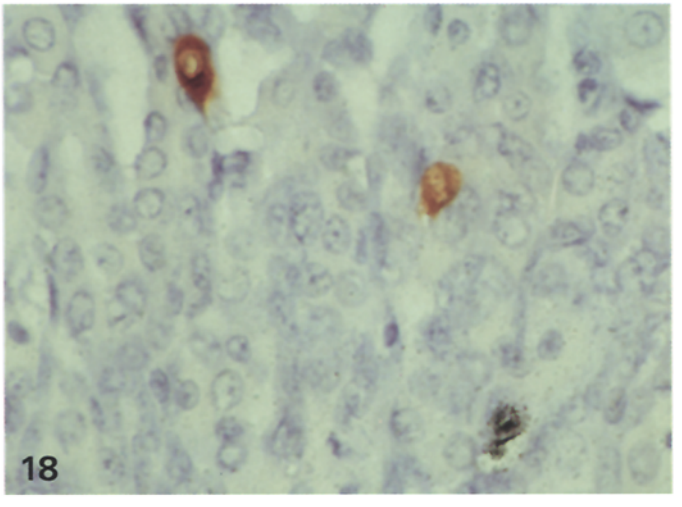
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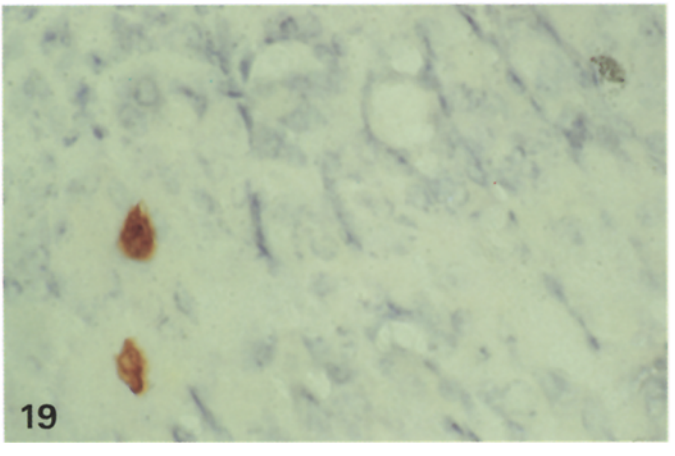
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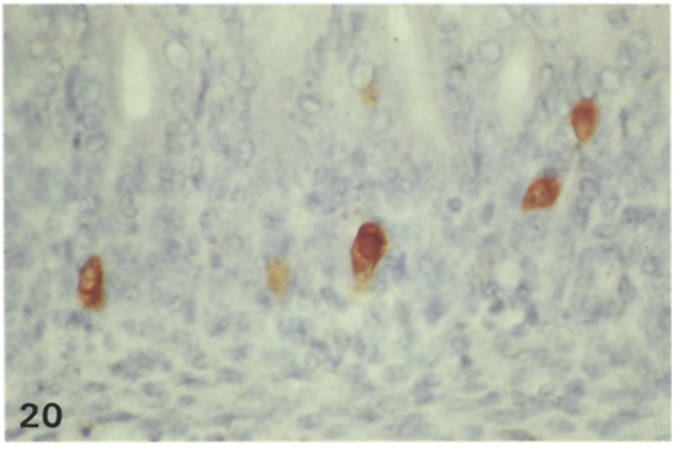
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statin-like cells (Figs. 13–14). Quantification of these cells showed that the population of somatostatin-like cells able to take up GABA represents 16.1% ± 4.27% (95% confidence limits (c.l.); 46/285) of immunoreactive cells at 2.5 days and 14.7% ± 2.64% (95% c.l.; 101/688) at 7.5 days.

Sections treated with anti-gastrin antibodies displayed a less frequent and lower autoradiographic labelling in the antral area containing immunoreactive cells than in non-immunoreactive epithelial regions. Control experiments performed in the absence of primary antibody or by preincubating it with the homologous antigen, showed no immunolabelling.

Discussion

Immunocytochemical and autoradiographic localization of GABA-immunoreactivity and high-affinity GABA uptake

The raising and the specificity of the antiserum directed against GABA-glutaraldehyde has been described elsewhere (Seguela et al. 1984). This antiserum has already been used with success in previous experiments on rat pancreas and duodenum (Gilon et al. 1988). In the present study, the specificity of the immunocytochemical reaction was confirmed on brain and stomach sections either by omitting the primary antibody or by preincubating the antibody in the presence of the GABA- and β-alanine-glutaraldehyde reduced conjugates.

The presence of GABA-immunoreactivity and GABA uptake in endocrine cells of the rat stomach is in good agreement with other studies, revealing immunostaining and autoradiographic labelling in endocrine cells of the duodenum and epithelial cells of the gastro-intestinal tract (Gilon et al. 1987b, 1988; Jessen et al. 1988; Davanger et al. 1989). The majority of these cells in the gastric epithelium lack any contact with the lumen of the glands. The fact that GABA-immunoreactivity was heterogeneous in different nuclei could result from differential accessibility of the nuclear antigenic sites to the antibodies in the thick Vibratome sections. The GABA-immunoreactivity in the nucleus correlates with the autoradiographic labelling in this structure and with experiments performed on nerve cells, pancreas and duodenum with a similar antiserum (Gamrani et al. 1986; Gilon et al. 1988).

Figs. 13–20. Paraffin sections of stomach fragments incubated in the presence of ^{3}H-GABA and processed for the immunocytochemical detections of somatostatin (**13–16**), cholecystokinin (**17–18**) and gastrin (**19–20**) coupled with autoradiography. A brown deposit revealed by the PAP reaction is seen on cells detected by immunocytochemistry whereas silver grains appears on cells detected by autoradiography. **Figs. 13–16.** The presence of silver grains on two of the somatostatin-like cells (*arrows*) reveals that a subpopulation of them has taken up ^{3}H-GABA. (**Figs. 13–15.** 7.5-day-old rat; **Fig. 16.** 2.5-day-old rat). **Figs. 17–20.** No overlapping of both labellings can be detected as well on cholecystokinin-as on gastrin-like cells (**Figs. 17–18, 20.** 7.5 day-old rat; **Fig. 19.** 2.5 day-old rat). **13** × 825; **14** × 900; **15** × 725; **16** × 930; **17** × 600; **18** × 650; **19** × 500; **20** × 440

Identification of the endocrine cell types interacting with GABA

Electron microscopic analysis. Although the electron microscope did not allow to identify the nature of the hormones, several endocrine cell types could be suggested to interact with GABA on the simple basis of their granular morphology: D, D_1, or X cells (Grube and Forssmann 1979; Solcia et al. 1975, 1981, 1987). The A-like, EC, ECL, and G cells can be excluded because the characteristic morphology of their granules does not correspond to that observed in the labelled cells (Grube and Forssmann 1979; Solcia et al. 1975, 1981, 1987; Rubin and Schwartz 1983, 1984). Nevertheless, even if typical EC cells with pleiomorphic secretory granules were never seen marked with high-resolution autoradiography, it is not excluded that other cells containing serotonin could perhaps interact with GABA. As a matter of fact, neurons containing both serotonin and GABA have already been reported in the central nervous system (Nanopoulos et al. 1982; Gamrani et al. 1984).

Whatever possibility it could be, the use of coupled methods are helpful in solving this problem. However, the double immunodetection of GABA and hormonal peptides was found to be impracticable with our anti-GABA antibody. GABA immunodetection in the stomach only succeeds when using a minimum concentration of 2% glutaraldehyde in a fixative free of the components of Bouin's liquid. This fixative does not retain the antigenicity of many peptides. By contrast, the coupled immunodetection of hormonal peptides and autoradiographic localization of GABA uptake was found feasible.

Immunodetection of hormonal peptides coupled with autoradiographic localization of GABA uptake. In order to test several peptide antisera through this coupled method, a fixative ensuring both conservation of antigenicity and retention of ^{3}H-GABA in the tissue had to be settled. Several mixtures of fixatives were then tested and the use of 2% glutaraldehyde added to Bouin's liquid was found to be the most appropriate combination for the coupled method. Bouin's liquid was the best fixative for the immunodetection of hormonal peptides, and glutaraldehyde was reported to be essential to avoid any diffusion of small molecules, such as GABA, in the tissue (Gamrani et al. 1984).

The use of this coupled method allowed to partially confirm the previous assumption based on the ultrastructural characteristics of the secretory granules that certain somatostatin-like cells (D cells) are able to take up GABA, contrarily to gastrin-like cells (G cells). The CCK-39-immunoreactive cells probably correspond to gastrin cells since the CCK-39 antiserum is able to detect mainly the sulfated form of gastrin G-17 and since no cholecystokinin cells were reported to be present in the stomach mucosa (Larsson 1977; Socia et al. 1981).

On the basis of the distribution of the gastrin-immunoreactive cells which have been reported to be present only in the antral mucosa (Larsson 1977; Solcia et al. 1981), the autoradiographic labelling was found to be more intense and frequent in the oxyntic than in the antral area. The weak autoradiographic labelling on cells dispersed between the gastrin-immunoreactive cells could be attributed to somatostatin cells. By contrast, the autoradiographic labelling in the oxyntic mucosa could correspond to at least two types of cells, somatostatin and X or D_1 cells, since the

majority of the grain-labelled cells are not immunoreactive in sections treated with anti-somatostatin antibodies. The assumption that X or D_1 cells could also be implicated in the uptake of GABA correlates both with the ultrastructural characteristics of the secretory granules, and with the distribution of these cells which have been reported to be consistently present only in the oxyntic mucosa (Solcia et al. 1981).

It is not known if GABA immunoreactivity and uptake are shared by identical or different populations of endocrine cells. The coupling of autoradiographic detection of ^{3}H-GABA with GABA-immunodetection could help to solve this problem. Several experiments were performed in this way and overlapping of both stainings was observed (Kisvarday et al. 1986; Jessen et al. 1988). Nevertheless, the respective contribution of endogenous or taken up GABA to the immunocytochemical labelling was difficult to assess. The granular morphology seems to indicate that both abilities could be shared by common populations since certain cell types possessing similar granules (350–400 nm) were labelled by the one or the other technique. In many cases however, the granules of the grain-labelled cells were quite smaller (250 nm) than those of the immunoreactive cells, suggesting that cells taking up GABA are not necessarily those containing it. This possibility is supported by the example of the endocrine pancreas: the insulin cells possess high level of GABA but are unable to take it up, whereas the somatostatin cells possess a high affinity uptake of GABA but do not contain it (Okada 1986; Gilon et al. 1988, 1989). In this view, immunocytochemistry against the enzymes of synthesis (GAD) or degradation (GABA-T) of GABA coupled to autoradiography would be helpful in solving this problem.

Functional implications of interactions between GABA and endocrine cells

On basis of these morphological observations, several hypotheses concerning the physiological role(s) of GABA in relation with the endocrine cells can be suggested.

(1) The labelled cells (either by GABA-immunodetection or autoradiography) may be a target of GABA which could originate from different sources: neighbouring epithelial cells (endocrine?), GABAergic innervation which is now well established in the stomach (Hills et al. 1987; Jessen et al. 1987; Davanger et al. 1987) or vascular/interstitial source.

The observation of a high-affinity uptake of GABA in somatostatin cells of the stomach is not unique as similar results were described in somatostatin cells of the pancreas (Gilon and Remacle 1989) and the duodenum (unpublished results). This uptake seems to be correlated to a physiological role of GABA which has been reported to decrease somatostatin release by rat pancreatic islets, antral mucosa and whole stomach (Taniguchi et al. 1982; Harty and Franklin 1983, 1986; Harty and Murthy 1986; Guo et al. 1989). The plasma somatostatin-like immunoreactivity was also diminished during infusion of GABA in dogs or after treatment of humans with sodium valproate, a GABA transaminase inhibitor (Kusunoki et al. 1988; Thirlby et al. 1988). Whether such effects are mediated by an action on receptors, carriers or other mechanisms is not yet known. However, a recent study has revealed the presence of specific $GABA_A$ receptors in the mucosal layer of the rat stomach (Erdö et al. 1989). Direct GABA-somatostatin interaction has also been reported in neurosecretory cells of the anterior periventricular hypothalamic area and in the endocrine pancreas of rat (Taniguchi et al. 1982; Kakucska et al. 1988).

The reason why certain somatostatin cells are unable to take up GABA is unclear. It could perhaps coincide with the physiological state of the cell or the degree of cell maturity. Technical failures could hardly been invoked to explain this heterogeneity.

As previously suggested, GABA can also interact with non-somatostatin cells. This is again correlated with physiological studies reporting that GABA stimulates as well the release of gastrin by the rat antral mucosa as the release of secretin by the duodenal mucosa (Harty and Franklin 1983, 1986; Harty and Murthy 1986). Nevertheless, it was proposed that these effects were mostly mediated by an indirect way via the cholinergic innervation rather than by a direct action on these cells.

(2) The immunolabelled cells (possibly able of GABA reuptake) can be a source of GABA. In preliminary experiments, we could reveal GAD-immunoreactivity in endocrine-like cells of the rat gastric mucosa suggesting that a GABA synthesis in situ exists in these cells. If GABA is released, it could act on other cell types and play an endocrine/paracrine role as already suggested for the endocrine pancreas (Garry et al. 1987a; Gilon et al. 1988, 1989). It would be an indirect way for it to control the endocrine secretion or the functional state of other neighbouring cells. A non-neuronal, releasable GABA pool has been observed in the rat gastric antrum (Erdö and Wolff 1988). A role of GABA in gastric acid secretion and ulcer formation, by central or peripheral receptors, has also been suggested (Lloyd et al. 1986; Minano et al. 1987; Thirlby et al. 1988).

(3) GABA may play an intracellular role: it could constitute an alternative energy source by the GABA-shunt or a similar pathway or it could be transformed and/or integrated in the formation of a peptide.

(4) Finally, it can not be excluded that GABA uptake or GABA-immunoreactivity represent characters of neuroendocrine cells such as the APUD ability, the transient tyrosine hydroxylase expression, the presences of neuron specific enolase and protein P 38, ... (Teitelman et al. 1981; Navone et al. 1986; Le Douarin 1988). Whether they are simply remnants of a still contested neuroectodermic origin of endocrine cells or reflect a real physiological role, is not yet clear. With this in view, it is interesting to report that all the immature cells of the rat fetal endocrine pancreas are able to take up GABA whereas this uptake is restricted only to somatostatin cells in adults (Gilon et al. 1987a).

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