Histochem Cell Biol (1997) 108:105–113

ORIGINAL PAPER

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Immunoelectron microscopic study of the luminal release of serotonin from rat enterochromaffin cells induced by high intraluminal pressure

Accepted: 15 January 1997

Abstract Since definitive morphological studies showing the luminal release of serotonin have not been reported, we used a perfused system which allows physiological monitoring and biochemical as well as morphological evidence indicating release of serotonin from enterochromaffin cells. Isolated vascularly and luminally perfused rat duodenums exposed to 5–35 cmH_2O of luminal pressure were measured for release of serotonin into the blood vessels and intestinal lumen. Immediately after raising the luminal pressure, the duodenum was fixed for immunoelectron microscopic localization of serotonin. Peristaltic contraction and serotonin content of the perfusates were continuously measured. The luminal release of serotonin increased with elevated intraluminal pressure, but the vascular release of serotonin was not altered. Tetrodotoxin had no effect on the pressure-stimulated luminal serotonin release. Enterochromaffin cells in control animals without increased luminal pressure contained immunogold-labeled secretory granules in the apical and basal cytoplasm. After intraluminal pressure increased, many apical secretory granules were no longer dense and immunogold particles were localized over the cytoplasmic matrix and microvilli. These findings indicate that luminal serotonin release is increased after raising the intraluminal pressure and serotonin, normally stored in the secretory granules of enterochromaffin cells, appears to be released into the cytoplasmic matrix and then diffuses or is transported into the intestinal lumen.

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Introduction

Enterochromaffin (EC) cells in the digestive tract have been considered to release their secretory granules across the basal cell membrane into the blood vessels to exert endocrine actions on distant targets. However, a number of in vivo studies have shown a measurable amount of serotonin (5HT) in the intestinal lumen of various species. Known stimulants for such luminal release of 5HT include vagal nerve stimulation (Ahlman et al. 1981; Grönstad et al. 1987; Zinner et al. 1982), luminal acidification (Kellum et al. 1983; Resnick and Gray 1962), food intake (Ferrara et al. 1987) or an increase in luminal pressure (Bülbring and Crema 1959). Luminal release of 5HT has also been examined in vitro using isolated mucosa/submucosal sheets, and known stimulants include mucosal acidification and cholinergic and β-adrenergic mechanisms (Forsberg and Miller 1982, 1983; Kellum et al. 1983, 1984a, b). However, there is no convincing morphological evidence demonstrating the luminal release of 5HT and it is still controversial whether the presence of 5HT in the intestinal lumen is actually the result of exocrine release from intestinal EC cells. A previous study has shown a bipolar distribution of secretory granules in the EC cell of normal rat duodenum (Nilsson et al. 1987), however, the number of granules found in the apical cytoplasm was too small to allow an unequivocal interpretation of the exocrine secretion of 5HT.

In the present study we demonstrate morphological evidence supporting the luminal release of 5HT from the EC cell by electron microscopy of isolated vascularly and luminally perfused rat duodenal preparations. Luminal release of 5HT was stimulated by increased intraluminal pressure and the duodenal segment was fixed for immunohistochemical study. These preparations permit the observation of morphological changes under stimulated luminal release. Comparable ultrastructural changes in the density of the secretory granules of gastric antral G cells (Forssmann and Orci 1969; Forssmann et al. 1980; Håkanson et al. 1982; Track et al. 1978) and D cells (Lamberts et al. 1991) have been reported with,

however, no coincident demonstration of ultrastructural intracellular immunoreactivities. As shown in our previous studies, the morphological change of the secretory granules was not always consistent with the change of intracellular localization of GI hormones (Fujimiya et al. 1995b; Okumiya et al. 1996). In this study the labeling of immunogold demonstrates the intracellular localization of 5HT immunoreactivity within EC cells.

Materials and methods

Measurement of 5HT release from isolated vascularly and luminally perfused rat duodenum

Male Wistar rats weighing 250–300 g were used. Animals were housed in a light-controlled room with free access to laboratory food and water, but were fasted overnight (16–18 h) before the experiment. Animals were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg Nembutal; Abbott Laboratories, USA). The duodenal segment lying between the pylorus and the ligament of Treitz was prepared for in situ vascular and luminal perfusion as described previously (Fujimiya et al. 1992). All blood vessels supplying both kidneys and adrenal glands, the stomach, pancreas, spleen, large intestine, and the rest of the small intestine were excluded from the perfusion. The stomach, pancreas, spleen, small and large intestine were carefully separated from the duodenal segment and removed. Arterial perfusion was achieved by inserting a cannula (P 160 tubing) into the aorta with its tip lying close to the junction of the celiac and superior mesenteric arteries. The aorta above the celiac junction was tied off. The vascular effluent was collected through a portal vein cannula. Luminal perfusion was through a cannula in the pylorus and the effluent was collected by a cannula in the duodenum at the ligament of Treitz. Intraluminal pressure was measured by a catheter inserted from the pylorus with the tip in the mid-portion of the duodenum. The catheter was connected to a transducer (TP-400T; Nihon Koden, Japan) and the pressure was amplified by an amplifier (AP-601G; Nihon Koden). Intraluminal pressure was continuously monitored and recorded on a polygraph (RM-6100; Nihon Koden).

The vascular perfusate was Krebs' solution containing 3% dextran and 0.2% bovine serum albumin (RIA grade; Sigma Chemical, USA). The perfusate was saturated with 95% O_2/5% CO_2 gas to maintain a pH of 7.4. The luminal perfusate was 0.1 M phosphat-buffered saline (PBS, pH 7.4). Both perfusates and the preparation were kept at 37° C throughout the experiment by thermostatically controlled heating apparatus. The flow rate for vascular and luminal perfusion was 3 ml/min and 1 ml/min, respectively. After a 25-min equilibration period, both vascular and luminal effluents were collected a 3-min samples for 33 min in collecting scintillation vials kept at 4° C. At perfusion period 5, the intraluminal pressure was raised by clamping the luminal effluent and the clamp was released when pressure reached the appropriate level. Three pressures, low (5–10 cmH_2O, n=7), medium (15–20 cmH_2O, n=5), and high (25–35 cmH_2O, n=4), were used. In some experiments (n=4), tetrodotoxin (TTX; Sankyo, Japan) was introduced into the vasculature via a side-arm infusion catheter (0.1 ml/min) to achieve a final concentration of 1 μM during periods 4–7 when the intraluminal pressure at period 6 was high (25–35 cmH_2O).

The determination of 5HT was by HPLC. For each ml of effluent, 10 μl of 57 mM ascorbic acid, 10 μl of 10 mM disodium EDTA, 10 μl of 1 M perchloric acid, and 10 μl of 51 mM pargyline hydrochloride were added. Vascular effluents were filtered with Ultrafree-MC (30000 NMWL; Nihon Millipore, Japan) by centrifuging for 30 min at 10000 rpm at 4° C. Luminal effluents were filtered manually with a 0.22-μm pore disk filter (Millex-GV; Nihon Millipore). Aliquots of 100 μl were injected into the HPLC and 5HT content was measured (Fujimiya et al. 1991). Results were expressed as mean±SEM ng/ml in each fraction. Statistical analysis of the data shown in Fig. 2 was by a single factor ANOVA for repeated measurements followed by the Dunnet t-test. A paired t-test (two-tail) was used to compare the value of mean basal release and the value in period 5 shown in Table 1.

Immunoelectron microscopic study of EC cells

Immediately after exposure to high (25–35 cmH_2O, n=2) or low (5–10 cmH_2O, n=2) intraluminal pressure, duodenal segments were vascularly perfused for 5 min with cold fixative containing 4% paraformaldehyde, 0.5% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer (PB, pH 7.4). For controls (n=2), perfused duodenums without clamping were fixed. After fixation, the duodenal segment was excised and postfixed for 24 h at 4° C in postfixative containing 4% paraformaldehyde and 0.2% picric acid in 0.1 M PB at 4° C. The duodenums were washed for 4 days with several changes of 0.1 M PB containing 15% sucrose and then soaked overnight in 0.1 M PB containing 10% gelatin at 37° C. The gelatin-embedded specimens were cooled to 4° C, fixed for 3 h with 4% paraformaldehyde in 0.1 M PB at 4° C, cut into 50-μm thick sections on a vibratome, and collected in 0.1 M PBS at 4° C.

The sections were pretreated for 10 min with a mixture of 0.01% trypsin (Sigma Chemical) and 0.68 mM $CaCl_2$ in 0.05 M TRIS-HCl buffer (pH 7.6) at 20° C. The sections were washed for 30 min with 0.1 M PBS and incubated for 48 h with antiserum against 5HT (Fujimiya et al. 1986) diluted 1:10000 in 0.1 M PBS at 4° C. To inactivate endogenous peroxidase activity, the sections were incubated at room temperature (RT) for 20 min with 0.1% H_2O_2 in 0.1 M PBS followed by an additional 20 min with 0.1% phenylhydrazine in 0.1 M PBS. This combined use of H_2O_2 and phenylhydrazine is more powerful than single use and does not affect the bound primary antibodies. After washing for 30 min with 0.1 M PBS, the sections were incubated at RT for 2 h in biotinylated anti-rabbit IgG (Vector Laboratories, USA) diluted 1:1000 in 0.1 M PBS. They were washed and placed for 2 h in avidin-biotin-peroxidase complex (Elite; Vector) diluted 1:2000 in 0.1 M PBS at RT. Immunoreaction was then rendered visible by reacting with 0.05 M TRIS-HCl buffer (pH 7.6) containing 0.01% 3,3′-diaminobenzidine (DAB), 1% ammonium nickel sulfate, and 0.0003% H_2O_2 for 30 min at RT.

The DAB-nickel-stained sections were washed for 10 min with 0.1 M PBS and for an additional 10 min with distilled H_2O. The sections were dehydrated by a graded series of ethanol as described in our previous paper (Okumiya et al. 1996). The sections were incubated overnight with LR gold resin (London Resin, UK) at −20° C and incubated for 1 h with LR gold resin containing 0.1% Benzil (Pelco; Ted Pella, USA) at −20° C. The sections were then mounted on silicon-coated glass slides and embedded in LR gold resin containing 0.1% Benzil and then covered with silicon-coated cover glasses at −20° C. The embedded specimens were polymerized for 4 h in an ultraviolet cryo chamber (Pelco; Ted Pella), where the temperature was kept at −20° C throughout the polymerization. After removal of the cover glasses, the polymerized resin sections were observed under light microscopy (×40) and areas which contained DAB-positive cells were cut with a razor blade. Ultrathin sections were cut in an ultramicrotome (Ultracut E; Reichert-Jung, Austria). The ultrathin sections were picked up on the nickel grids (thin bar grid, 200-mesh; Nishin EM, Japan) supported by collodion.

The nickel grids were incubated for 30 min at RT with 3% normal goat serum (Dako Japan, Japan) dissolved in a reaction buffer containing 0.2% BSA, 0.2% saponin, and 0.05% NH_4Cl in 0.1 M PBS. The grids were incubated for 2 h at RT with antiserum against 5HT diluted 1:300 in the reaction buffer. The sections were washed with 0.1 M PBS and incubated for 1.5 h with immunogold-conjugated goat anti-rabbit IgG (15-nm gold; British BioCell International, UK) diluted 1:40 at RT. The sections were washed with 0.1 M PBS followed by distilled H_2O, then stained for 10 min with 2% uranyl acetate and for an additional 5 min with Reynolds' lead citrate solution at RT. During the above procedures, the nickel grids were floated with the sections exposed to the drops of incu-

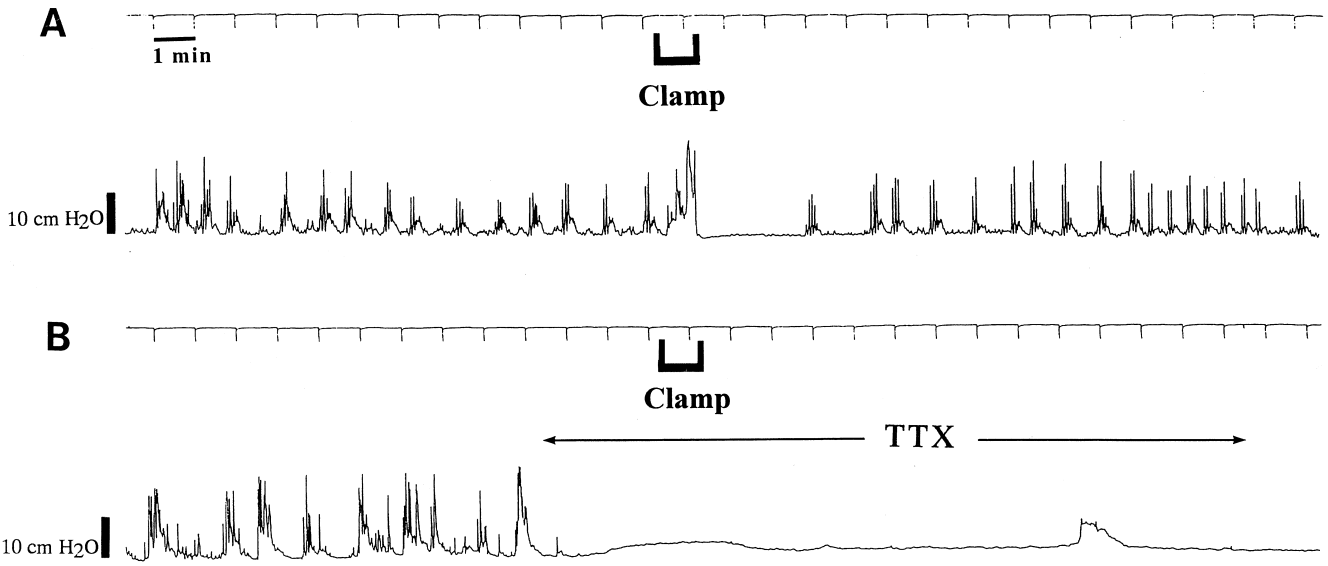

Fig. 1A, B Periodic contractions recorded in perfused rat duodenum by monitoring the intraluminal pressure. **A** When the luminal outflow is clamped, the baseline of the pressure curve is elevated. When the elevation of the baseline reaches the appropriate level (10 cmH_2O in this case), the clamp is released. The periodic contraction ceases for several minutes after releasing the clamp. **B** When 1 μM tetrodotoxin (*TTX*) is infused into the vasculature prior to elevation of intraluminal pressure, periodic contraction completely ceases

bation media or submerged in the washing media. The stained sections were then observed in the electron microscope (H-7100; Hitachi, Japan).

In the present study, more than 30 EC cells from control duodenums, more than 30 EC cells from the high intraluminal pressure group, and more than 20 EC cells from the low intraluminal pressure group were observed by electron microscopy. The specificity for the immunogold reaction was tested by a preabsorption study performed by postembedding staining on sections treated with low intraluminal pressure. The primary antibody was substituted with an antibody (diluted 1:300) preabsorbed with 100 μM 5HT. The antigen and antibody mixture was incubated for 48 h at 4° C before the immunostaining procedures.

Results

Periodic contractions were recorded in perfused rat duodenums by monitoring the intraluminal pressure during the experiment (Fig. 1A). The normal range of the peak amplitude of the contraction wave was between 0 and 25 cmH_2O. When the luminal outflow was clamped, the baseline of the pressure curve was elevated and it suddenly dropped when the clamp was released. Intraluminal pressure exerted on the duodenum was determined by the elevation of the baseline (Fig. 1A). Periodic contractions ceased for several minutes after elevation of the intraluminal pressure and then returned to their normal rhythm. However, contractions completely ceased when 1 μM TTX was infused into the vasculature of the perfused duodenum (Fig. 1B).

A consistent amount of 5HT was released into both intestinal lumen (1.84±0.17 ng/ml, n=16) as well as into the blood vessels (1.04±0.12 ng/ml, n=16) from isolated perfused rat duodenum. Raising the intraluminal pressure caused an increase of 5HT release into the lumen but did not affect the release of 5HT into the vasculature. Immediately after high (25–35 cmH_2O) luminal pressure

Table 1 The effect of high (25–35 cmH_2O, n=4), medium (15–20 cmH_2O, n=5) or low (5–10 cmH_2O, n=7) pressure on the release of serotonin into the lumen and the vasculature. Each value represents mean±SEM ng/ml

Pressure	Luminal release		Vascular release	
	Basal[a]	Stimulated[b]	Basal[a]	Stimulated[b]
High	1.26±0.30	3.90±0.63*	0.65±0.14	0.73±0.23
(% stimulation)		(248.5±78.7)		
Medium	1.97±0.39	3.58±0.96	1.36±0.36	1.28±0.43
(% stimulation)		(82.4±27.0)		
Low	2.07±0.18	3.57±0.80	1.13±0.13	1.11±0.26
(% stimulation)		(67.2±31.2)		

[a] Average of values during periods 1–4
[b] The value at period 5. Percentage stimulation is calculated as 100×(b−a)/a
* <0.05 compared to the basal release

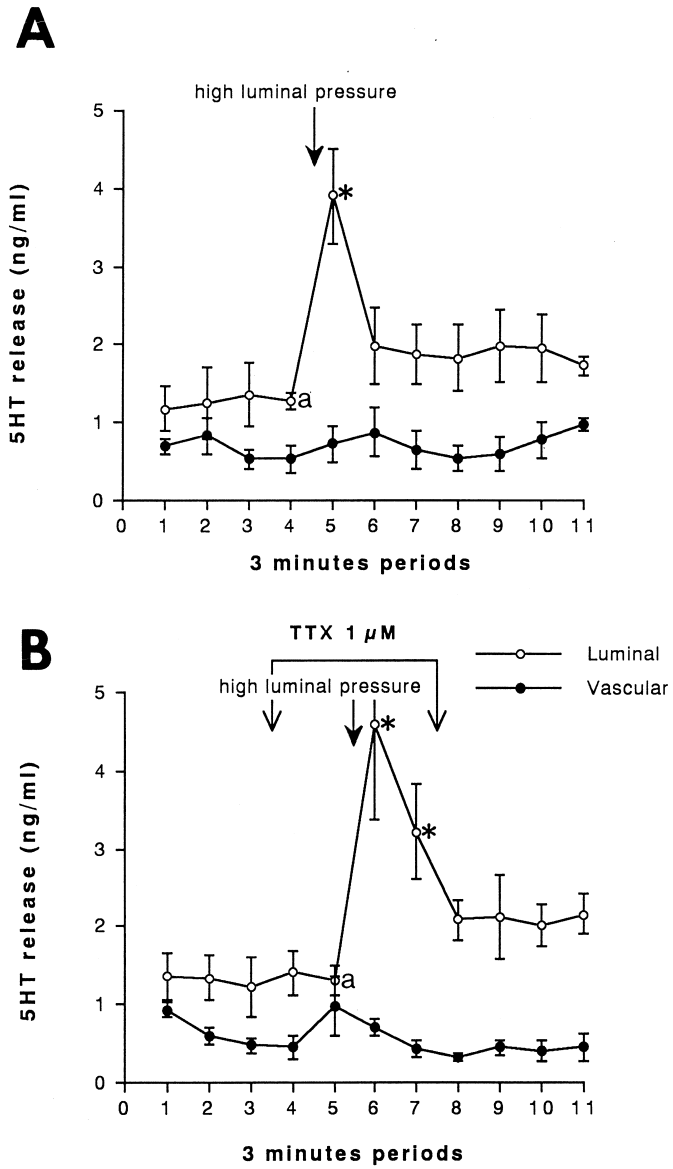

Fig. 2 The release of serotonin (*5HT*) into the portal circulation (●, *n*=4 in both **A** and **B**) as well as into the intestinal lumen (○, *n*=4 in both **A** and **B**) from isolated vascularly and luminally perfused rat duodenum. Each value represents mean±SEM ng/ml of 3-min samples. High intraluminal pressure (25–35 cmH_2O) was attained at times shown by the *arrows*. 5HT release levels marked with *asterisks* significantly increase when compared with the value marked *a*. TTX (1 μM) was infused during periods 4–7 (**B**). The stimulatory effect of luminal release of 5HT by high luminal pressure is not altered by TTX (**B**)

was attained, the luminal release (1.26±0.30 ng/ml, *n*=4) increased to 3.90±0.63 ng/ml (*n*=4) and returned to the basal level (1.89±0.37 ng/ml, *n*=4) after the luminal pressure was removed (Fig. 2A, Table 1). Similar patterns were observed with medium (15–20 cmH_2O) or low (5–10 cmH_2O) intraluminal pressure. However, the percentage stimulation of luminal 5HT release differed with different pressure levels (Table 1). The stimulatory effect of high intraluminal pressure on the luminal release of 5HT was not altered by TTX administered into the vasculature (Fig. 2B).

Electron microscopic observation of EC cells in control duodenums without luminal pressure revealed large aggregations of secretory granules in the apical as well as the basolateral cytoplasm, as shown in Fig. 3A. At higher magnifications, the apical cytoplasm demonstrated a number of small, round, electron-dense granules located beneath the microvilli (Fig. 3B). In some EC cells of control animals, relatively large granules were observed in the apical cytoplasm (Fig. 3D), which were comparable in size to the secretory granules in the basal cytoplasm shown in Fig. 3C. Some of the granules were situated very close to the apical plasma membrane (Fig. 3D). In control EC cells, immunogold label was concentrated over granules of both apical and basal plasma membrane but limited labeling was present over the cytoplasmic matrix (Fig. 3B–E).

Changes in EC cells stimulated with low intraluminal pressure are shown in Fig. 4A, B. Small and electron-dense granules aggregated in the apical cytoplasm, as in control duodenums, but immunogold particles were prominent over the apical cytoplasmic matrix and microvilli (Fig. 4A). In some EC cells there were many clear granules without cores and granules with eccentric cores in the apical cytoplasm (Fig. 4B). Immunogold reactions were located over granular cores and surrounding cytoplasmic matrix (Fig. 4B).

After high intraluminal pressure, the structure and the localization of 5HT immunoreactivity within the EC cells changed drastically. In some EC cells the apical cytoplasm expanded (Fig. 5A) or protruded into the lumen (Fig. 5C). The secretory granules situated in the apical cytoplasm were larger than those in the basal cytoplasm (Fig. 5A, B), and much larger than the secretory granules in the apical cytoplasm from control and low pressure groups. Therefore, the changes caused by high intraluminal pressure were more prominent in the apical than in the basal cytoplasm. There was heavy immunogold labeling of the cytoplasmic matrix and microvilli (Fig. 5). Secretory granules with cores and empty vesicles were in the apical cytoplasm below the terminal web, but immunogold particles were diffusely scattered over the terminal web and microvilli (Fig. 5A′, C). Only rarely were dense granules found near the apical cell membrane (Fig. 5A, A′).

The specificity for the immunogold reactions was tested by using antibody preabsorbed with 5HT on sections treated with low intraluminal pressure (Fig. 4C). Since the sections were treated with the DAB-nickel reaction prior to embedding, EC cells were readily identified by electron microscopy. Immunogold labeling was completely negative, but granules with and without cores were present at the apical cytoplasm (Fig. 4C).

Discussion

Previous reports indicated that increased luminal pressure caused measurable release of 5HT from EC cells in a loop of guinea pig ileum (Bülbring and Crema 1959; Bülbring and Lin 1958). In these experiments, relatively

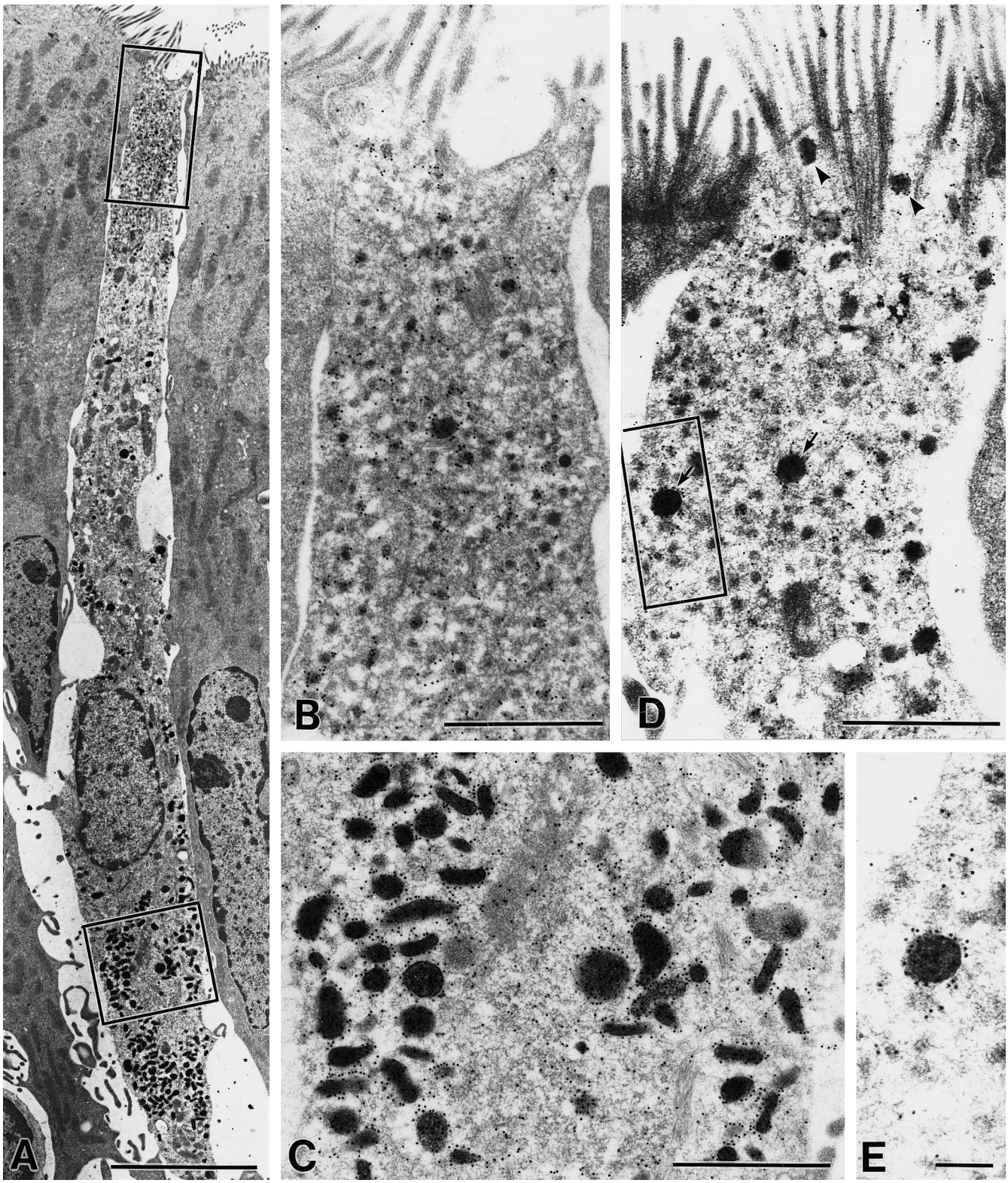

Fig. 3A–E Electron micrographs of enterochromaffin (EC) cells in control duodenum. **A** Aggregation of secretory granules in the apical as well as basolateral cytoplasm. *Bar* 5 μm. **B** Higher magnification of the apical cytoplasm enclosed by the *rectangle* in **A**. A number of small, round, electron-dense granules located beneath the microvilli can be seen. Immunogold label is concentrated over these granules. *Bar* 1 μm. **C** Higher magnification of the basal cytoplasm enclosed by the *rectangle* in **A**. Electron-dense granules with pleomorphic shape are seen. Immunogold label is concentrated over these granules but limited labeling is present over the cytoplasmic matrix. *Bar* 1 μm. **D** Apical cytoplasm of another EC cell in control duodenum. Relatively large granules are seen (*arrows*) which are comparable in size to those in the basal cytoplasm seen in **C**. Some granules are situated very close to the apical plasma membrane (*arrowheads*). *Bar* 1 μm. **E** Higher magnification of the area enclosed by the *rectangle* in **D**. Immunogold label is concentrated over the dense granule. *Bar* 200 nm

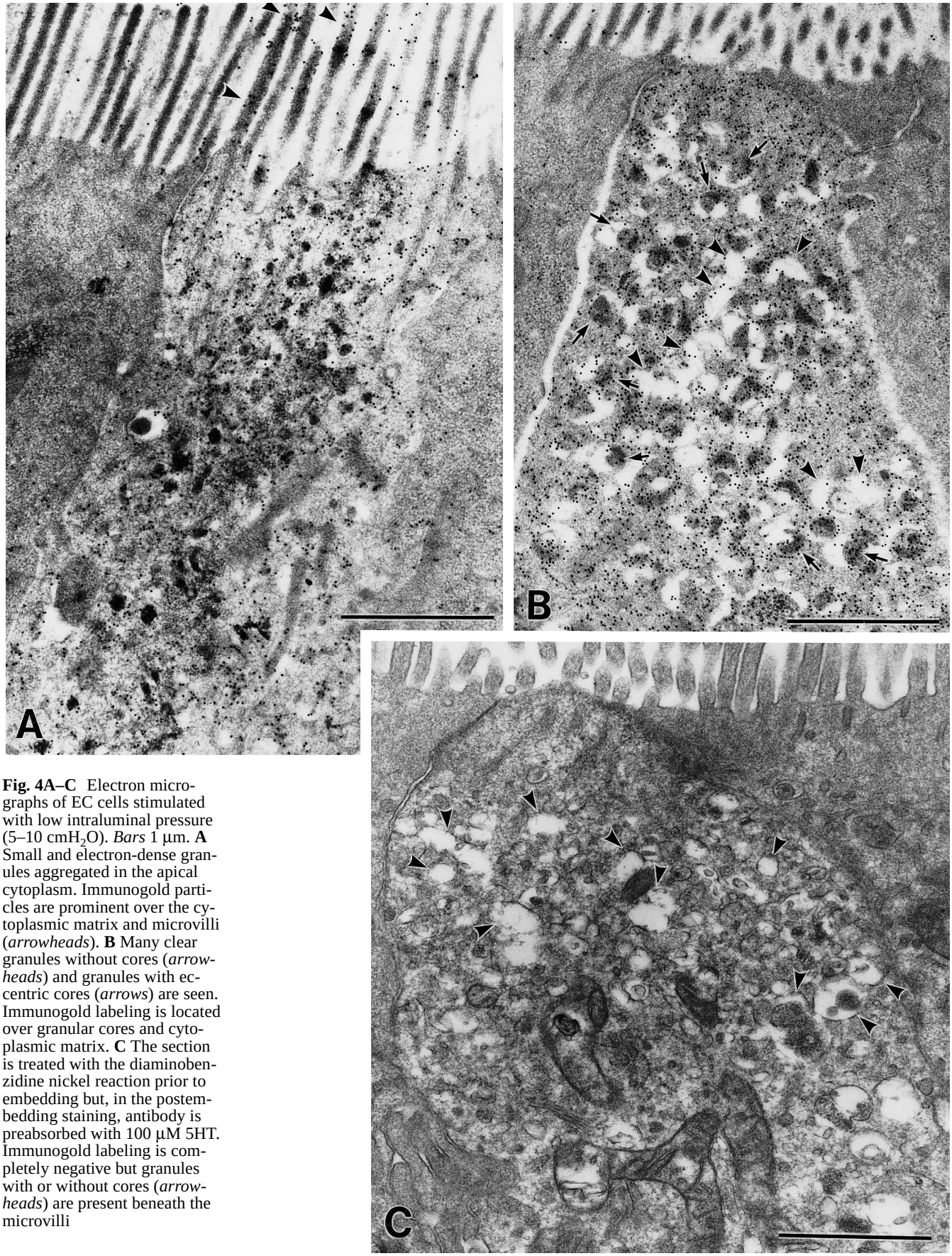

Fig. 4A–C Electron micrographs of EC cells stimulated with low intraluminal pressure (5–10 cmH_2O). *Bars* 1 μm. **A** Small and electron-dense granules aggregated in the apical cytoplasm. Immunogold particles are prominent over the cytoplasmic matrix and microvilli (*arrowheads*). **B** Many clear granules without cores (*arrowheads*) and granules with eccentric cores (*arrows*) are seen. Immunogold labeling is located over granular cores and cytoplasmic matrix. **C** The section is treated with the diaminobenzidine nickel reaction prior to embedding but, in the postembedding staining, antibody is preabsorbed with 100 μM 5HT. Immunogold labeling is completely negative but granules with or without cores (*arrowheads*) are present beneath the microvilli

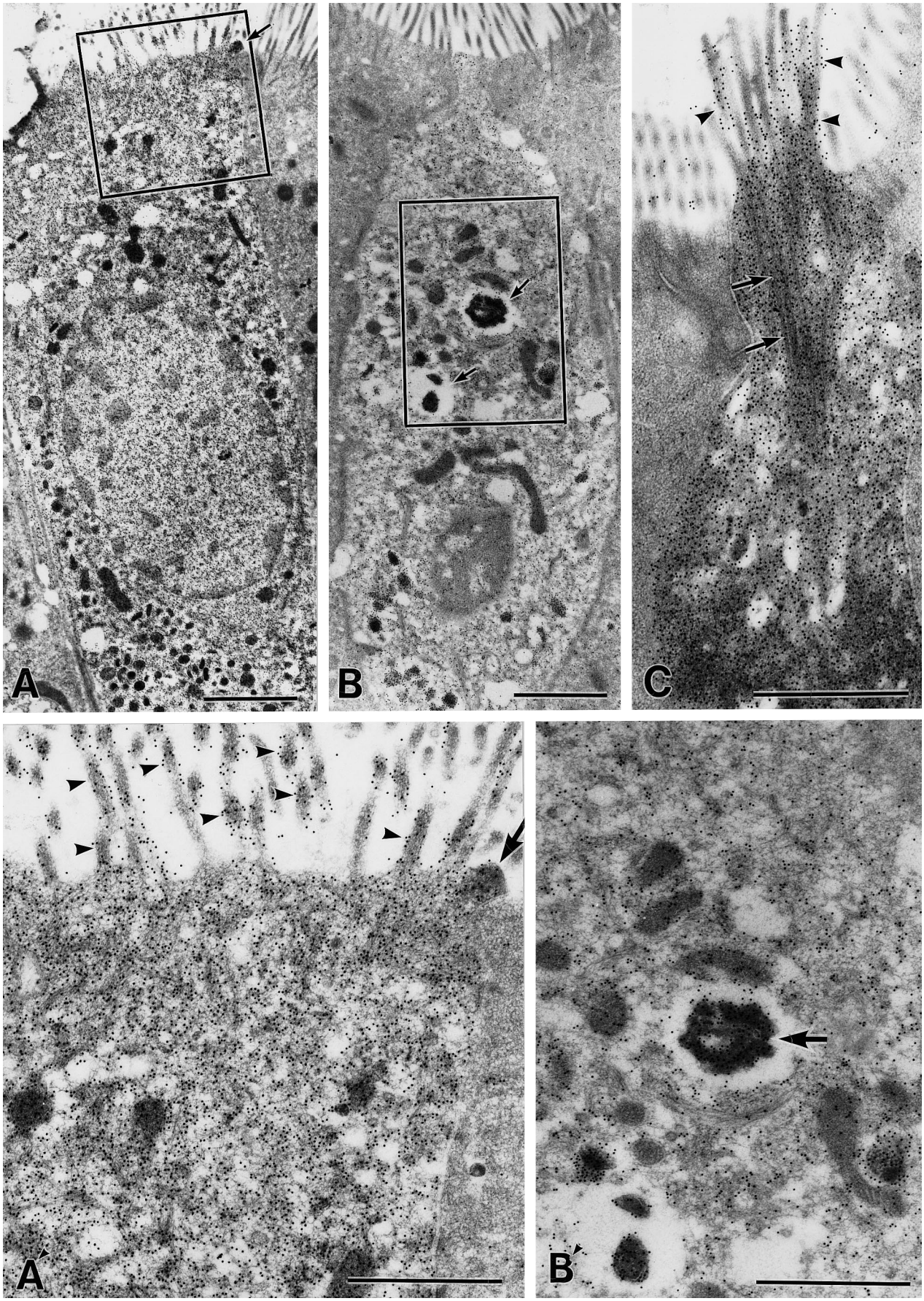

Fig. 5A–C, A′–B′

low pressure (less than 10 cmH_2O) was used on the mucosa, because the normal peristalsis in these isolated loops was less than 10 cmH_2O. In the present study, we used higher pressure levels because the peak amplitude of normal peristaltic contractions of perfused rat duodenum is 20–25 cmH_2O. As shown by our results, the release of 5HT into the lumen was increased significantly by raising luminal pressure, however, the release of 5HT into the vasculature was not altered. This increase of 5HT released into the lumen was not antagonized by TTX, suggesting that 5HT detected in the lumen might not be derived from 5HT-containing enteric neurons but from epithelial EC cells. Manometric measurements of the intraluminal pressure during the experiment revealed periodic contractions of the perfused duodenum. Complete cessation of these contractions by administration of TTX suggests that motor activity of perfused duodenum was mediated by neuronal transmission within the duodenal wall.

The viability of the isolated perfused rat intestine was demonstrated in our previous study (Fujimiya et al. 1995a). The present study revealed that most of the EC cells in control duodenum retained their normal ultrastructure and contained secretory granules in the apical and basal cytoplasm. We found that the number of granules in the apical cytoplasm was much higher than previously reported in rat EC cells (Nilsson et al. 1987).

In the present study, we used the postembedding immunogold method combined with the preembedding DAB-nickel reaction using antibody against 5HT in both immunohistochemical procedures. This combined method appears very useful for cells such as intestinal endocrine cells, which are scattered sparsely in the intestinal epithelium, because the cells that are considered for subsequent immunogold electron microscopy can be previously selected under light microscopy. The present results showed rich immunogold reactions remained even though the sections were processed for preembedding DAB-nickel staining. In our previous study, we compared the density of immunogold particles over the secretory granuls in sections previously stained with DAB and those without DAB staining (Okumiya et al. 1996). As a result, the density was not significantly changed between the two groups. Therefore, the preembedding staining does not interfere with the immunoreactivity for postembedding staining but serves as a good landmark for locating immunopositive cells.

Diffuse distribution of immunogold labeling in the cytoplasmic matrix and the increase of empty granules observed in stimulated EC cells may not be an artifact caused by poor fixation or perfusion preparations of the duodenal tissues because a different distribution of immunogold labeling, such as concentration over cores of the secretory granules, was found in control duodenums treated with the same fixation or perfusion methods, although only without high intraluminal pressure.

The ultrastructure of EC cells and the intracellular distribution of immunoreactive 5HT changed remarkably after intraluminal pressure was increased. In control animals, immunogold particles were concentrated over secretory granules with little labeling of the cytoplasmic matrix. Such extragranular localization of 5HT has been reported in EC cells of normal rat intestine (Nilsson et al. 1985). When low pressure was applied to the duodenum, immunogold labeling of the apical cytoplasm increased and was mostly scattered over the matrix. After high intraluminal pressure, more prominent changes such as an increase of empty granules, swelling of secretory granules, and the protrusion of plasma membrane into the lumen appeared. The size of the secretory granules in the basal cytoplasm was similar in most EC cells of both control and stimulated animals, however, that in the apical cytoplasm varied considerably in response to high luminal pressure. Furthermore, in EC cells under high luminal pressure, massive aggregation of the immunogold particles was observed in the apical cytoplasmic matrix and over the microvilli. Since the HPLC data showed that the luminal release of 5HT incresed with elevated intraluminal pressure, such ultrastructural changes described above may indicate a morphological feature of exocrine release of 5HT from EC cells. Under normal conditions, 5HT is primarily stored in the EC cell secretory granules. However, in response to the intraluminal pressure, 5HT appears to be released into the extragranular matrix and released into the lumen through the apical cell membrane.

Endocrine cells in the digestive tract have been demonstrated to release their secretory granules from the basal cell membrane by exocytosis (Kobayashi and Fujita 1973). The present study shows that luminal exocytosis of secretory granules is very rare, but diacrine release of secretory granule content is extensive. These findings resemble previous observations that most gastrin granules in antral G cells appear empty after stimulation of gastrin release, however, changes in the intracellular localization of gastrin have not been shown (Forssmann and Orci 1969; Forssmann et al. 1980; Håkanson et al. 1982; Track et al.1978). Our previous study, on the other hand, has shown the changes in intracellular localization of immunoreactive gastrin in duodenal G cells after carbachol stimulation, in which empty granules increased in the apical cytoplasm and immunogold particles were abundant in the extracellular matrix (Okumiya et al. 1996). Our previous study has also shown the intracellu-

◀ **Fig. 5A–C, A′, B′** Electron micrographs of EC cells stimulated with high intraluminal pressure (25–35 cmH_2O). **A** Apical cytoplasm expands and the granules in the apical cytoplasm are larger than those in the basal cytoplasm. Heavy immunogold labeling is seen over the cytoplasmic matrix and microvilli. *Bar* 2 μm. **A′** Higher magnification of the area enclosed by the *rectangle* in **A**. A dense granule is found near the apical cell membrane (*arrow*). Immunogold particles are diffusely scattered over the terminal web and microvilli (*arrowheads*). *Bar* 1 μm. **B** Remarkably large granules with or without cores are found in the apical cytoplasm (*arrows*). *Bar* 2 μm. **B′** Higher magnification of the area enclosed by the *rectangle* in **B**. Immunogold labeling is concentrated over the granular core (*arrow*). *Bar* 1 μm. **C** Immunogold particles are diffusely scattered over the terminal web, well developed microtubules (*arrows*), and microvilli (*arrowheads*). *Bar* 1 μm

lar localization of 5HT in embryonic EC cells; we observed protrusion of apical cell membranes and diffuse localization of immunogold particles in the cytoplasmic matrix (Fujimiya et al. 1995b). Biochemical analysis also detected considerable amounts of 5HT in the lumen of embryonic gut (Fujimiya et al. 1995b).

The physiological role of luminally released 5HT has been proposed in previous studies. Mucosal application of 5HT or its precursor, 5-hydroxytryptophan, increased peristaltic movement of isolated loops of guinea pig ileum (Bülbring and Crema 1959; Bülbring and Lin 1958).

Endoluminal application of 5HT caused the hyperemia of cat intestinal segment and this response was prevented by local anesthesia applied to the luminal surface (Grönstad et al. 1986; Zinner et al. 1982). The functional significance, however, of luminally released 5HT from EC cells is not understood completely. The present results show that 5HT was released from EC cells in response to an increase in intraluminal pressure within the physiological range. These findings suggest that 5HT released into the lumen may play an important role in intestinal functions such as absorption, secretion or movement of the intestine.

Acknowledgements This study was supported by a Grant-In-Aid for Scientific Research (C) from the Ministry of Education, Science, Sports and Culture, Japan (M.F.). The authors thank Mr. Takefumi Yamamoto in the Central Research Laboratory of Shiga University of Medical Science for his excellent technical support in the electron microscopic study.

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