Histol Histopathol (1999) 14: 453-460

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Changes in intestinal endocrine cells in the mouse after unilateral cervical vagotomy

B.-F. Qian[1], M. El-Salhy[1], Å. Danielsson[1], A. Shalaby[3] and H. Axelsson[2]

[1]Section for Gastroenterology and Hepatology, Department of Medicine, [2]Department of Surgery, University Hospital, Umeå, Sweden and [3]Department of Zoology, Faculty of Science, University of Zagazig, Zagazig, Egypt

Summary. The effect of right or left unilateral cervical vagotomy on the intestinal endocrine cells was studied in 23 mice at 2 and 8 weeks after operation, respectively. The results were compared with that from 10 sham operated mice. Various types of endocrine cells in duodenum and proximal colon were detected by immunohistochemistry and quantified by computerized image analysis. In mouse duodenum, chromogranin-, CCK/gastrin-, GIP- and somatostatin-cells were significantly decreased at 2 weeks after right vagotomy, but returned to the control levels at 8 weeks. Serotonin-cells were reduced at both 2 and 8 weeks after right vagotomy. The amount of the duodenal endocrine cells did not change after left vagotomy with the exception of secretin-cells, which were diminished at 8 weeks after both right and left vagotomy. In the proximal colon, chromogranin-cells were also decreased at 2 weeks after right vagotomy. Serotonin-cells were reduced at 8 weeks after left vagotomy but not right vagotomy. There was no significant difference between the unilaterally vagotomized and the sham operated mice with regard to PYY- and glucagon-cells. It was concluded that vagotomy affected the intestinal endocrine cells in mouse. The influence was more pronounced in the small intestine than the proximal colon. The right vagus nerves seemed to exert more effect on the intestinal endocrine cells than the left ones.

Key words: Computerised image analysis, Endocrine cells, Intestine, Mouse, Unilateral vagotomy

Introduction

The parasympathetic innervation of oesophagus, stomach, small intestine and proximal colon is supplied by the vagus nerves. The parasympathetic activity interacts and integrates with the gastrointestinal neuroendocrine system to regulate several important functions of the gut, such as secretion, motility, and blood flow (Qian et al., 1996).

Vagotomy, due to its inhibitory effect on gastric acid secretion, has in the past been used as an effective treatment for patients with peptic ulcer disease. Vagal disturbance is also a common complication in some gastrointestinal diseases (Lindgren et al., 1993; El-Salhy et al., 1994), and may also contribute to the development of diseases. With the emerging concept of multifactorial regulation of the gut, and with the increasing evidence for a close relationship between the vagus nerves and the gut neuroendocrine system both in their structures and functions (Debas and Mulvihill, 1991; Lindgren et al., 1993; Qian et al., 1996), there is a growing interest with respect to the impact of the vagus nerves on the endocrine cells of the gut. Several morphological studies have been carried out over the years to investigate the effect of the vagus nerves on the endocrine cells in antrum (Tobe et al., 1976; Arnold et al., 1982; Magallanes et al., 1982; Portela-Gomes, 1982; Håkanson et al., 1984; Pederson et al., 1984; Holle et al., 1985a,b; Mulholland et al., 1985; Inman et al., 1990; Koop et al., 1993). In contrast, very few studies have been performed to investigate the impact of vagotomy on the endocrine cells in other parts of the gut (Tobe et al., 1976; Izumikawa, 1980; Portela-Gomes, 1982).

The present study was undertaken to investigate the possible changes in the intestinal endocrine cells in mouse, both after a short term and after a long term observation time after right and left cervical vagotomy, respectively.

Materials and methods

Animals

Studies were performed on 33 male mice (BOM: NMRI Strain, B/S Bomholtgård Breeding Research Centre, Denmark), aged 3 months and with an average body weight of 30 g. The animals were kept in a temperature-controlled and air-conditioned room with artificial light and dark cycles of 12 hours, and fed with

Offprint requests to: Magdy El-Salhy, MD, PhD, Section for Gastroenterology and Hepatology, Department of Medicine, University Hospital, S-901 85 Umeå, Sweden. Fax: +46-90-143986. e-mail: magdy.el-salhy@medicin.umu.se

a standard pellet diet (Astra-Ewos AB, Södertälje, Sweden) and water ad libitum. They were housed in our vivarium 2 weeks before operation.

This investigation was approved by the local committee on animal ethics, Umeå University.

Surgical procedures

After overnight fast, the animals were unilaterally vagotomized. They were anaesthetised with a mixture of midazolam and fentanyl/fluanson. They were randomly divided into 3 groups: 12 subjected to left vagotomy, 11 subjected to right vagotomy and 10 subjected to sham operation as controls. In the vagotomy groups, left or right vagal trunks were identified and sectioned at the level of the neck, in close contact with the carotid artery. A minimum length of 5 mm of the nerves was resected and histological examination was performed to confirm the identification. The incision was closed with skin sutures and the animals were allowed to recover. In the sham operation group, the surgery was performed in the same way but the vagus nerves were left intact.

After a 2-week period of observation following the operation, 17 mice were randomly chosen from the three groups (5 from the sham operation group, 6 from each of the two vagotomy groups) and sacrificed in a CO_2-chamber. The rest of the mice were killed at 8 weeks after the operation.

Immunohistochemical technique

Immediately after sacrificing the animals, the proximal duodenum and proximal colon were dissected out, fixed in 4% phosphate buffered formaldehyde overnight and embedded in paraffin. Five-micrometer sections were cut from the specimens. For immunohistochemical demonstration of the endocrine cells, the avidin-biotin complex (ABC) method was used as described in details elsewhere (El-Salhy et al., 1993). The following primary polyclonal rabbit antisera were used: chromogranin AB, cholecystokinin (CCK)/gastrin, gastric inhibitory polypeptide (GIP), secretin, serotonin, somatostatin, glucagon, polypeptide YY (PYY) and pancreatic polypeptide (PP). A detailed account of the antisera used is given in Table 1.

Negative controls were obtained by using non-immune rabbit serum in place of the primary antibodies, by preincubating the primary antibodies with an excess of the corresponding or structurally related antigens (75-100 μg/ml diluted antibodies) for 24 hours at 4 °C, or by substitution of the secondary antibody with non-immune rabbit serum. Positive controls were obtained by processing the sections from human duodenum and colon.

Computerised Image analysis

Morphometric analysis of the endocrine cells in duodenum and proximal colon was performed as described previously (El-Salhy et al., 1997). This was done by using Quantimet 500 MC image processing and analysis system (Leica, Cambridge, England) connected to a microscope (type BX50, Olympus, Japan). The number of the endocrine cells with visible nuclei was counted by using manual field measurement. The area of the epithelial cells was measured by using threshold setting. To measure chromogranin AB-, CCK/gastrin-, GIP-, and serotonin-immunoreactive cells in duodenum, 50 fields were randomly chosen from 3 sections (at least 80 μm apart from each other) from each animal, 25 fields from the villi and 25 fields from the crypts of mucosa. For secretin-immunoreactive cells, 25 fields from the villi were examined, whereas 25 fields from the crypts were analysed for somatostatin-immunoreactive cells in duodenum. To investigate chromogranin AB-, serotonin-, glucagon- and PYY-immunoreactive cells in proximal colon, 25 fields randomly chosen from 3 sections were quantified for each mouse.

The sections were examined with a x20 objective and each field seen in the monitor represented 0.034 mm^2 area of tissue. For each mouse, the average height of villi, and the average number of villi and crypts corresponding to 1 mm baseline of mucosa in duodenum was determined. The area of epithelium corresponding to 1 mm baseline of mucosa in proximal colon was also measured. It was done by the same image analysis

Table 1. A detailed account of the antisera used

ANTIBODIES RAISED AGAINST	WORKING DILUTION	CODE no	SOURCE
Chromogranin AB	1:100	R2716-B7	Euro-Diagnostica, Malmö, Sweden
Synthetic gastrin 17*	1:2500	R783511-B5	Euro-Diagnostica, Malmö, Sweden
Porcine GIP	1:1000	R786403-B2	Euro-Diagnostica, Malmö, Sweden
Porcine secretin	1:200	R787502-B33-1	Euro-Diagnostica, Malmö, Sweden
Serotonin	1:1000	R871204-B4	Euro-Diagnostica, Malmö, Sweden
Synthetic somatostatin	1:2000	A566	Dakopatts, Glostrup, Denmark
Porcine glucagon**	1:1000	R781101-B3	Euro-Diagnostica, Malmö, Sweden
Synthetic polypeptide YY	1:1000	R841303-B4	Euro-Diagnostica, Malmö, Sweden
Synthetic PP	1:500	A619	Dakopatts, Glostrup, Denmark

CCK: cholecystokinin; GIP: gastric inhibitory polypeptide; PP: pancreatic polypeptide; *: Specific for CCK/gastrin C-terminus; **: crossreacts with pancreatic glucagon and enteroglucagon.

system using a 950 x 950 μm^2 frame and a x4 objective. Three fields chosen from three sections cut perpendicular to the mucosa surface for each specimen were examined. All measurements were carried out by one investigator.

Statistical analysis

Results are presented as mean±SEM. Differences between the groups were analysed by the non-parametric Wilcoxon rank sum test. A p-value less than 0.05 was considered to be statistically significant.

Results

Immunohistochemistry

Endocrine cells immunoreactive to the antibodies against chromogranin AB and serotonin were identified in both duodenum and proximal colon. Cells immunoreactive to the antibodies against CCK/gastrin, GIP, secretin, and somatostatin were only demonstrated in the duodenum. PYY- and glucagon-immunoreactive cells were identified in the proximal colon, while PP- and somatostatin-immunoreactive cells were seldom encountered. These immunoreactive cells were observed in both sham-operated and vagotomized mice. The cells varied in shape, including flask-shaped cells with a narrow apical process towards the lumen and basket-shaped cells with a basal process towards the adjacent epithelial cells. Immunoreactive secretory granules were more often seen around the nuclei, and in the basal part of cells.

In the mouse duodenum, chromogranin AB-, CCK/gastrin-, GIP-, and serotonin-immunoreactive cells were observed in both villi and crypts. Secretin-immunoreactive cells were mainly localised in the villi, whereas somatostatin-immunoreactive cells were mostly found in the crypts.

Specificity controls showed that replacement of the primary antibodies by non-immune rabbit serum or preincubation of the antibodies with the corresponding peptides resulted in no immunostaining, which was also the case after substitution of the secondary antibody with

Table 2. The number of various endocrine cells in 1 mm^3 epithelial cells in the villi and/or crypts of duodenum in the sham-operated and the unilaterally vagotomized mice at different observation times after operation

		2 weeks			8 weeks		
		S	L	R	S	L	R
Cg AB	Villus	4114±561	5515±572	1975±624 *	3901±866	2355±1099	1811±650
	Crypt	7076±1434	9948±964	3080±703 *	9674±2311	9486±4534	6526±903
	Total	11190±1494	15463±1430	5055±1316 *	13575±3091	11841±4638	833 7±979
CCK/Gastrin	Villus	1934±363	2365±326	1278±141	1680±265	1971±271	2974±412
	Crypt	2982±549	3833±552	845±142 **	2814±554	1524±309	2090±579
	Total	4916±591	6198±609	2123±242 **	4494±707	3494±530	5064±862
GIP	Villus	1580±136 †	1747±282	270±100 **	704±250	333±125	698±282
	Crypt	2430±485 †	2098±881	186±84 **	734±248	790±310	861±190
	Total	4010±541 ††	3845±1098	456±169 **	1438±203	793±191	1558±372
Secretin	Villus	692±216	1593±313	502±153	1856±517	202±153 **	534±50 **
Serotonin	Villus	6712±322 †	5455±410	3177±218 **	4540±458	2269±718	2666±491 *
	Crypt	14276±2366	12455±862	7673±1317 *	8926±1456	5498±1352	3962±1139
	Total	20988±2451	17910±1051	10850±1363 *	13466±1591	7768±1797	6628±1236 *
Somatostatin	Crypt	2562±380 †	2755±384	1098±215 **	852±309	82±82	1340±482

S: sham operation; L: left vagotomy; R: right vagotomy; Cg AB: chromogranin AB; CCK: cholecystokinin; GIP: gastric inhibitory polypeptide. Values are expressed as mean±SEM. *: p<0.05; **: p<0.01, versus the sham-operated mice. †: p<0.05; ††: p<0.01, the sham-operated mice 2 versus 8 weeks after operation.

Table 3. The number of various endocrine cells in 1 mm^3 epithelial cells of colon in the sham-operated and the vagotomized mice

	2 weeks			8 weeks		
	S	L	R	S	L	R
Cg AB	15870±3452	14938±2342	6086±1621 *	11298±1535	6420±1271	5700±1726
Serotonin	9460±1796	11837±1739	10564±506	13220±1130	6643±904 **	11654±2219
Enteroglucagon	6244±1619	7080±1327	4262±874	5414±399	4722±671	5302±968
PYY	3768±841	5283±812	2086±651	3030±547	2618±513	3500±556

S: sham operation; L: left vagotomy; R: right vagotomy. Cg AB: chromogranin AB; PYY: polypeptide YY. Values are expressed as mean±SEM. *: p<0.05; **: p<0.01, versus the sham-operated mice.

non-immune rabbit serum. Preincubation of the primary antibodies with structurally related peptides had no effect on the immunostaining. Treatment of the sections from human duodenum and colon with the antibodies gave positive staining.

Computerised image analysis

The results of morphological measurements of various duodenal and colonic endocrine cells in the sham-operated and the vagotomized mice are

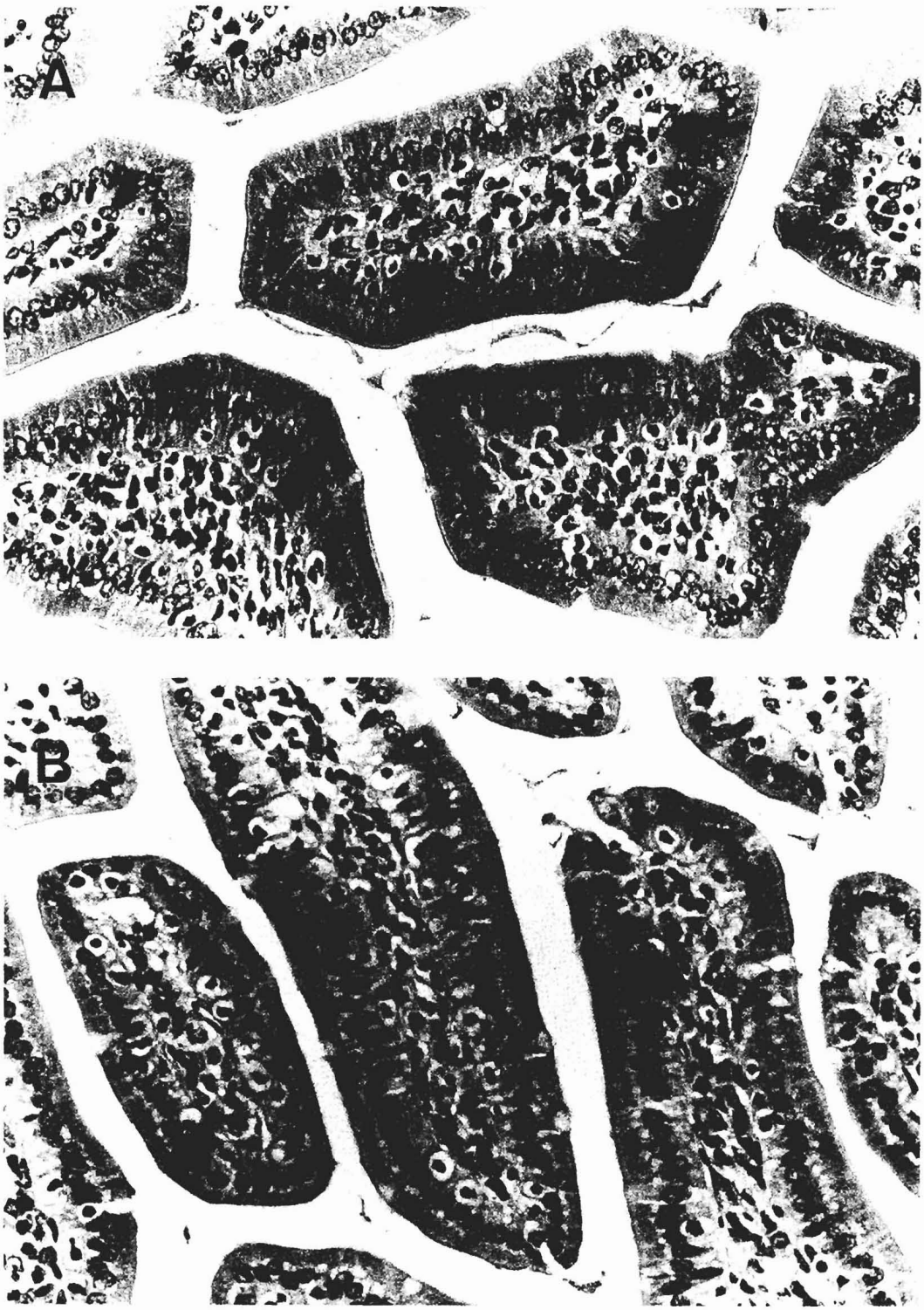

Fig. 1. Serotonin-immmunoreactive cells in the villi of duodenum of a mouse 2 weeks after right cervical vagotomy **(A)**, and of a mouse after sham operation **(B)**. The amount of serotonin-immunoreactive cells was reduced in the vagotomized mouse as compared to the sham-operated one. x 400

Table 4. Average height of villi, and average number of villi and crypts corresponding to 1 mm baseline of duodenal mucosa (mean±SEM) in the sham-operated and the vagotomized mice.

	2 weeks			8 weeks		
	S	L	R	S	L	R
Height of villi (μm)	435.9±26.5	390.1±21.7	356.1±17.4	352.4±47.4	418.5±16.4	422.4±19.2
Number of villi	9.8±0.9	10.6±0.6	10.4±1.0	11.3±0.6	11.4±0.4	12.5±0.6
Number of crypts	34.7±4.7	29.4±0.6	35.1±2.6	32.7±2.1	28.6±1.6	28.5±2.2

S: sham operation; L: left vagotomy; R: right vagotomy.

Table 5. The epithelium area (μm^2) corresponding to 1 mm baseline of colon (mean±SEM) in the sham-operated and the vagotomized mice.

	2 weeks	8 weeks
Sham operation	178.4±36.2	170.0±25.1
Left vagotomy	199.9±29.2	164.5±17.3
Right vagotomy	127.9±6.1	147.5±11.4

summarised in Tables 2-3.

Duodenum

The number of chromogranin AB-immunoreactive cells was significantly decreased in the mice 2 weeks but not 8 weeks after right vagotomy as compared to the controls. This reduction was observed both in the villi and in the crypts. No statistically significant changes were found in the mice after left vagotomy at any time of the observation period.

The numbers of CCK/gastrin-, and GIP-immunoreactive cells were both reduced in the mice 2 weeks after right vagotomy as compared to the control mice after sham operation. The reduction in the number of CCK/gastrin-immunoreactive cells was mainly found in the crypts, while the reduction of GIP-immunoreactive cells was observed in both villi and crypts. At 8 weeks after right vagotomy, these changes returned to the control levels. There were no significant changes in these two types of endocrine cells in the mice after left vagotomy. With respect to the GIP-immunoreactive cells, there was a significant increase in the mice at 2 weeks after sham operation as compared to those after 8 weeks, both in the villi and in the crypts.

Secretin-immunoreactive cells did not change at 2 weeks after unilateral cervical vagotomy, but were markedly decreased in the right as well as the left vagotomized mice at 8 weeks after the operation.

In the animals subjected to right vagotomy, there was a significant decrease in the number of serotonin-immunoreactive cells, both 2 and 8 weeks after the operation (Fig 1). This reduction could be observed in the villi and/or the crypts. However, no significant differences were found between the mice after left vagotomy and the mice after sham operation at any time during the experiment. A trend towards increase in serotonin-immunoreactive cells was found in the mice 2 weeks after sham operation as compared to the mice after 8 weeks. This change was significant for the villi.

Decreased number of somatostatin-immunoreactive cells was noted in the mice at 2 weeks after right vagotomy as compared to the controls. However, no such a change was found in the mice at 8 weeks after right vagotomy and in the mice at any time point after left vagotomy. In the mice 2 weeks after sham operation, somatostatin-immunoreactive cells were increased as compared to those after 8 weeks.

Proximal colon

Chromogranin AB-immunoreactive cells were decreased in the mice at 2 weeks after right vagotomy and returned to the control level after 8 weeks, whereas no change was found in the mice subjected to left vagotomy. The number of serotonin-immunoreactive cells was reduced in the mice at 8 weeks after left vagotomy. However, there was no significant change in the mice after right vagotomy as compared to the controls. With regard to the amount of PYY- and glucagon-immunoreactive cells, no statistically significant differences were observed between the unilaterally vagotomized and the sham operated mice at any time after the operation.

Mucosa

The average height of villi, the average number of villi and crypts corresponding to 1 mm baseline of duodenal mucosa in the sham operated and the vagotomized animals, are summarised in Table 4. There was no statistically significant difference between the groups. Neither was any significant change found in the epithelial area of proximal colon in the unilaterally vagotomized mice as compared to the controls (Table 5).

Discussion

The results from the present study indicated that vagotomy had a marked effect on the intestinal endocrine cells in mouse. The influence was more pronounced on the small intestine than the proximal colon. However, this was not unexpected, because there is less innervation of the vagus nerves to the distal part

than to the proximal part of the intestine. The right vagus nerves seemed to be more essential in maintaining the normality of the intestinal endocrine cells than the left ones. All types of duodenal endocrine cells included in this study were decreased after right vagotomy, whereas only secretin-immunoreactive cells were changed after left vagotomy. These results could be explained by the fact that parasympathetic innervation in duodenum is supplied mainly by the posterior vagal trunk, which is mainly composed of the right vagus nerve. The alterations of the intestinal endocrine cells after vagotomy in this study could be classified as subacute and chronic. The subacute alterations seemed to be more prominent. Most of the changes in the endocrine cells occurred at 2 weeks after the operation. However, they were usually short-lasting and disappeared after 8 weeks. In contrast, chronic alterations found 8 weeks post-operatively were few.

Chromogranin has been claimed to be a general marker for the gastrointestinal endocrine cells (O'Connor et al., 1983; Facer et al., 1985; Eriksson et al., 1990). However, other investigators have demonstrated that chromogranin is only co-localised with a few types of peptides/amines in rat and green frog (Bargsten and Grube, 1992; D'Este et al., 1994). Furthermore, it has been reported that chromogranin immunoreactivity in peptide-producing cells varies among species, among gastrointestinal segments and even in the same endocrine cell type (Ceting et al., 1989). It seems, therefore, that chromogranin-positive cells are not representative for the whole population of endocrine cells. In the present study, the amount of chromogranin AB-immunoreactive cells was even less than that of serotonin-immunoreactive cells in mouse duodenum.

Similar to our recent finding on the antral endocrine cells, where a reduction of chromogranin AB-immunoreactive cells was found in the mice after unilateral vagotomy (Qian et al., 1999), the number of chromogranin AB-immunoreactive cells was also decreased in both small and large intestine 2 weeks after right vagotomy. However, the decrease of chromogranin AB-immunoreactive cells in antrum was delayed to 4-8 weeks after the surgery. This time discrepancy may be explained by the differences in kinetic behaviours of the endocrine cells in different sites of the gut. The half-life of endocrine cells in antrum has been estimated to 10-15 days, while the turn over time of endocrine cells in duodenum was only 2-4 days (Inokuchi et al., 1983). Since the alterations of the endocrine cells after vagotomy are time dependent, the selection of time for observation is important when performing quantitative studies on the gut endocrine cells.

The effect of vagotomy on gastrin-immunoreactive cells in antrum has been extensively studied (Magallanes et al., 1982; Pederson et al., 1984; Mulholland et al., 1985; Inman et al., 1990; Koop et al., 1993). To the best of our knowledge, however, there has been no report on the density of gastrin-immunoreactive cells in duodenum (the second largest population of gastrin-cells) after vagotomy. The reduction of CCK/ gastrin-immunoreactive cells reported here reflected the changes in both CCK- and gastrin-cells, as the antiserum used is specific for CCK/gastrin C-terminus.

While previous studies agreed on the hypergastrinemia after vagotomy, the effect of vagotomy on extragastric gastrin release was controversial. Either unchanged (Emås and Fyrö, 1965; Malmstrom et al., 1977; Hughes, 1986) or increased (Korman et al., 1972; Booth et al., 1975; Eckhard et al., 1978) secretion of duodenal gastrin was reported from the observations made on feline, dog, and human. Some differences in the characteristics of duodenal gastrin release as opposed to antral gastrin release have also been reported (Wesdrop et al., 1977). That could probably be related to the different types of gastrin molecules. Duodenal gastrin has a different prevailing molecular form as compared to antral gastrin, i.e. big gastrin (G-34) constitutes a higher proportion of duodenal gastrin than of antral gastrin (Malmstrom et al., 1976). In contrast, CCK release was not under the vagal control (Fried et al., 1983; Singer et al., 1989; Lundell et al., 1991).

The amounts of secretin- as well as GIP-immunoreactive cells were decreased after unilateral cervical vagotomy. Endogenous secretin release from duodenum was reported to be beyond the regulation of the vagus nerves (Soloman and Grossman, 1977; Chey et al., 1979; Niebel et al., 1988). However, equivocal results were obtained concerning the function of the vagus nerves on GIP-immunoreactive cells. It was reported that the basal plasma GIP level was elevated (Yoshiya et al., 1985), or was not influenced by vagotomy (Imamura et al., 1984).

Serotonin-immunoreactive cells were decreased in both duodenum and proximal colon after unilateral vagotomy in this study. The effect of vagotomy on the intestinal serotonin-cells has been an issue of controversy. It has been reported that the serotonin level increased (Tobe et al., 1976; Izumikawa, 1980) or did not change (Reichle et al., 1970, 1971) in duodenum, or decreased in duodenum and colon (Portela-Gomes et al., 1985) after vagotomy in rat. These differences could be explained by the differences in observation time, in surgical procedure (with or without pyloroplasty), and in methods for measurement.

The number of duodenal somatostatin-immunoreactive cells was significantly decreased after right vagotomy. Somatostatin-cells are well known to play an inhibitory role on the gastrointestinal endocrine cells including gastrin-, CCK-, secretin-, and GIP-cells by a paracrine mode of action (Mulvihill and Debas, 1994). The reduction of those cells seemed to be accompanied by a decrease in somatostatin-immunoreactive cells.

It might be worth mentioning that the numbers of GIP- and somatostatin-immunoreactive cells in duodenum were increased in the mice 2 weeks after sham operation. These changes may be related to post-operative stress and/or ageing.

Acknowledgements. This study was supported by grants from the Swedish Medical Research Council (19X-11240), the Medical Faculty Research Fund, Umeå University and the County of Västebotten.

References

Arnold R., Hülst M.V., Neuhof C.H., Schwarting H., Becker H.D. and Creutzfeldt W. (1982). Antral gastrin-producing G-cells and somatostatin-producing D-cells in different states of acid secretion. Gut 23, 285-291.

Bargsten G. and Grube D. (1992). Serotonin storage and chromogranins: an experimental study in rat gastric endocrine cells. J. Histochem. Cytochem. 40, 1147-1155.

Booth R.A.D., Reeder D.D. and Thompson J.C. (1975). Effect of antrectomy and subsequent vagotomy on the serum gastrin response to food in dogs. Ann. Surg. 181, 191-195.

Ceting Y., Muller-Koppel L., Aunis D., Bader M.F. and Grube D. (1989). Chromogranin A (CgA) in the gastro-entero-pancreatic (GEP) endocrine system II. CgA in mammalian entero-endocrine cells. Histochemistry 92, 265-275.

Chey W.Y., Kim M.S. and Lee K.Y. (1979). Influence of the vagus nerve on release and action of secretin in dog. J. Physiol. 293, 435-446.

D'Este L., Buffa R., Pelagi M., Siccardi A.G. and Renda T. (1994). Immunohistochemical localization of chromogranin A and B in the endocrine cells of the alimentary tract of the green frog, *Rana esculenta*. Cell Tissue Res. 277, 341-349.

Debas H.T. and Mulvihill S.J. (1991). Neuroendocrine design of the gut. Am. J. Surg. 161, 243-249.

Eckhard D.F., Grace M.D., Osborne M.P. and Fischer J.E. (1978). Lower esophageal sphincter pressure and serum gastrin levels after mapped antrectomy. Arch. Inter. Med. 338, 243-245.

El-Salhy M., Stenling R. and Grimelius L. (1993). Peptidergic innervation and endocrine cells in the human liver. Scand. J. Gastroenterol. 28, 809-815.

El-Salhy M., Suhr O., Stenling R., Wilander E. and Grimelius L. (1994). Impact of familial amyloid associated polyneuropathy on duodenal endocrine cells. Gut 35, 1413-1418.

El-Salhy M., Sandström O., Näsström E., Mustajbasic M. and Zachrisson S. (1997). Application of computer image analysis in endocrine cell quantification. Histochem. J. 29, 1-8.

Emås S. and Fyrö B. (1965). Vagal release of gastrin in cats following reserpine. Acta. Physiol. Scand. 63, 358-369.

Eriksson B., Arnberg H., Öberg K., Hellman U., Lundqvist G., Wernstedt C. and Wilander E. (1990). A polyclonal antiserum against chromogranin A and B - a new sensitive marker for neuroendocrine tumours. Acta. Endocrinol. (Copenh). 122, 145-155.

Facer P., Bishop A.E., Lloyd R.V., Wilson B.S., Hennessy R.J. and Polak J.M. (1985). Chromogranin: a newly recognized marker for endocrine cells of the human gastrointestinal tract. Gastroenterology 89, 1366-1373.

Fried G.M., Ogden W.D., Greeley G. and Thompson J.C. (1983). Correlation of release and actions of cholecystokinin in dogs before and after vagotomy. Surgery 93, 786-791.

Håkanson R., Vallgren S., Ekelund M., Rehfeld J.F. and Sundler F. (1984). The vagus exerts trophic control of the stomach in the rat. Gastroenterology 86, 28-32.

Holle G.E., Auerbach U., Höck H. and Holle F. (1985a). Changes of cell population in the antrum after selective proximal vagotomy and pyloroplasty in gastroduodenal ulcer. Surg. Gynecol. Obstet. 160, 211-219.

Holle G.E., Buck E., Pradayrol L., Wunsch E. and Holle F. (1985b). Behaviour of somatostatin-immunoreactive cells in the gastric mucosa before and after selective proximal vagotomy and pyloroplasty in treatment of gastric and duodenal ulcers. Gastroenterology 89, 736-745.

Hughes W.S. (1986). Duodenal gastrin concentration in upper gastrointestinal disorders. Dig. Dis. Sci. 31, 1201-1206.

Imamura M., Kameyama J., Naito H., Sato T. and Ohneda A. (1984). Influence of vagotomy upon GIP release in patients with peptic ulcer. Tohoku J. Exp. Med. 143, 335-344.

Inman L., Lee S.K., Shah I.A., Thirlby R.C. and Feldman M. (1990). Effect of truncal vagotomy on parietal cell mass and antral gastrin cell mass in dogs. Gastroenterology 99, 1581-1592.

Inokuchi H., Fujimoto S. and Kawai K. (1983). Cellular kinetics of gastrointestinal mucosa, with special reference to gut endocrine cells. Arch. Histol. Jpn. 46, 137-157.

Izumikawa F. (1980). Release mechanisms of 5-HT from the gastrointestinal tract in rats. Arch. Jpn. Chir. 49, 572-594.

Koop H., Frank M., Kuly S., Nold R., Eissele R., Rager G., Ruschoff J., Rothmund M. and Arnold R. (1993). Gastric argyrophil (enterochromaffin-like), gastrin, and somatostatin cells after proximal selective vagotomy in man. Dig. Dis. Sci. 38, 295-302.

Korman M.G., Soveny C. and Hansky J. (1972). Extragastric gastrin. Gut 13, 346-348.

Lindgren S., Stewenius J., Sjölund K., Lilja B. and Sundkvist G. (1993). Autonomic vagal nerve dysfunction in patients with ulcerative colitis. Scand. J. Gastroenterol. 28, 638-642.

Lundell L., Cantor P., Sjövall M., Rehfeld J.F. and Olbe L. (1991). Factors influencing the release of cholecystokinin induced by gastrin-releasing peptide in man. Scand. J. Gastroenterol. 26, 544-550.

Magallanes F., Quigley T.M., Mulholland M.W., Bonsack M. and Delaney J.P. (1982). Antral proliferation of G cells after truncal, parietal cell, and antral vagotomy. J. Surg. Res. 32, 377-381.

Malmstrom J., Stadil F. and Christensen K.C. (1977). Effect of truncal vagotomy on gastroduodenal content of gastrin. Br. J. Surg. 64, 34-38.

Malmstrom J., Stadil F. and Rehfeld J.F. (1976). Concentration and component pattern in gastric, duodenal, and jejunal mucosa of normal human subjects and patients with duodenal ulcer. Gastroenterology 70, 697-703.

Mulholland M.W., Bonsack M. and Delaney J.P. (1985). Proliferation of gastric endocrine cells after vagotomy in the rat. Endocrinology 117, 1578-1584.

Mulvihill S.J. and Debas H.T. (1994). Regulatory peptides of the gut. In: Basic and clinical endocrinology. 4th ed. Greenspan F.S. and Baxter J.D. (eds). Norwalk. Appleton & Lange. Stanford. pp 551-570.

Niebel W., Beglinger C. and Singer M.V. (1988). Pancreatic bicarbonate response to HCl before and after cutting the extrinsic nerves of the pancreas in dogs. Am. J. Physiol. 254, G436-443.

O'Connor D.T., Burton D. and Deftos L.J. (1983). Immunoreactive human chromogranin A in diverse polypeptide hormone producing tumors and normal endocrine tissues. J. Clin. Endocrinol. Metab. 57, 1084-1086.

Pederson R.A., Kwok Y.N., Buchan A.M.J., McIntosh C.H.S. and Brown J.C. (1984). Gastrin release from isolated perfused rat stomach after

vagotomy. Am. J. Physiol. 247, G248-G252.

Portela-Gomes G.M. (1982). Enterochromaffin cells in the rat gastrointestinal tract after vagotomy. Uppsala University.

Portela-Gomes G.M., Dahlström A., Grimelius L., Johansson H. and Ahlman H. (1985). The effect of truncal vagotomy on serotonin distribution in the rat gastrointestinal tract. J. Surg. Res. 38, 13-16.

Qian B-F., Danielsson Å. and El-Salhy M. (1996). Vagal regulation of the gastrointestinal neuroendocrine system. Scand. J. Gastroenterol. 31, 529-540.

Qian B-F., El-Salhy M., Danielsson Å., Shalaby A. and Axelsson H. (1999). Effects of unilateral cervical vagotomy on antral endocrine cells in mouse. Histol. Histopathol. (in press).

Reichle F.A., Goodman P.M., Brigham M.P., Reichle R.M., Labinsky L. and Rosemond G.P. (1970). Intestinal serotonin content following gastric resection or pyloroplasty with vagotomy. Arch. Surg. 101, 205-210.

Reichle F.A., Newcomer D.L., Brigham M.P., Reichle R.M., Labinsky L. and Rosemond G.P. (1971). The effect of vagotomy on the histamine content of the gastrointestinal tract. Arch. Surg. 103, 133-139.

Singer M.V., Niebel W., Jansen J.B.M.J., Hoffmeister D., Gotthold S., Goebell H. and Lamers C.B.H.W. (1989). Pancreatic secretory response to intravenous caerulein and intraduodenal tryptophan studies: before and after stepwise removal of the extrinsic nerves of the pancreas in dogs. Gastroenterology 96, 925-934.

Soloman T.E. and Grossman M.I. (1977). Cholecystokinin and secretin release are not affected by vagotomy or atropine. Gastroenterology 72, 1134.

Tobe T., Izumikawa F., Sano M. and Tanaka C. (1976). Release mechanisms of 5-HT in mammalian gastrointestinal tract - especially, vagal release of 5-HT. In: Endocrine gut and pancreas. 1st ed. Fujita T. (ed). Elsevier. Amsterdam. pp 371-380.

Wesdrop R.I.C., Funovics J.M., Hirsch H. and Fischer J.E. (1977). Characteristics of release of duodenal gastrin. Am. J. Surg. 133, 280-284.

Yoshiya K., Yamamura T., Ishikawa Y., Utsunomiya J., Takemura J., Takeda J., Seino Y. and Imura H. (1985). Effect of truncal vagotomy on GIP release induced by intraduodenal glucose or fat in dogs. Digestion 31, 41-46.

Accepted October 1, 1998