

## Effects of unilateral cervical vagotomy on antral endocrine cells in mouse

B-F. Qian<sup>1</sup>, M. El-Salhy<sup>1</sup>, Å. Danielsson<sup>1</sup>, A. Shalaby<sup>3</sup> and H. Axelsson<sup>2</sup>

<sup>1</sup>Section for Gastroenterology and Hepatology, Department of Medicine and <sup>2</sup>Department of Surgery, University Hospital, Umeå University, Sweden, <sup>3</sup>Department of Zoology, Faculty of Science, University of Zagazig, Zagazig, Egypt

**Summary.** The present study was carried out to investigate the effect of unilateral cervical vagotomy on the antral endocrine cells in mouse. Fifty-four mice were randomly divided into three groups, 18 in each, for left or right cervical vagotomy, or sham operation as controls. The animals were sacrificed 2, 4, and 8 weeks after the operation, respectively. Chromogranin-, gastrin/CCK-, serotonin-, and somatostatin-cells were detected by immunohistochemistry and quantitated by computerised image analysis. The results showed that the number of chromogranin-cells was decreased in both left and right vagotomized mice after 4 weeks and remained at the same level after 8 weeks. The numbers of gastrin-, serotonin- and somatostatin-cells did not change after right vagotomy. However, the numbers of gastrin- and somatostatin-cells were decreased after left vagotomy, whereas no change was found in serotonin-cells. Endocrine cells with vacuolated cytoplasm and pyknotic nuclei were also observed during the course of time. The alteration in the antral endocrine cells observed in this study seemed to be dynamic and depended on the observation time after the operation as well as the denervated branches of the vagus nerve. This may explain, at least partially the contradictory results obtained earlier by different investigators.

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

### Introduction

The vagus nerve constitutes an important link between the central nervous system and the gastrointestinal tract. Vagal stimulation promotes gastrointestinal motility and increases the secretion from gastrointestinal glands (Qian et al., 1996). Although much attention has been paid to the interaction between this nerve and the antral endocrine cells, much need yet to be clarified

(Qian et al., 1996). The number or density of gastrin-producing cells in antrum has been shown to be increased (Arnold et al., 1982; Magallanes et al., 1982; Koop et al., 1993; Holle et al., 1985a,b; Mulholland et al., 1985), or unchanged (Pederson et al., 1984; Inman et al., 1990) after bilateral truncal vagotomy or selective proximal vagotomy. Contradictory results have also been reported with regard to the changes in antral somatostatin- and serotonin-producing cells after vagotomy. While some studies have demonstrated increased staining for somatostatin- (Holle et al., 1985b; Mulholland et al., 1985) and serotonin-producing cells (Tobe et al., 1976; Izumikawa, 1980), others have found decreased numbers of these cells (Drapanas et al., 1971; Arnold et al., 1982; Koop et al., 1993). It has been suggested that these discrepancies might be due to the differences in species, anaesthesia and surgical procedures in various studies. Since the changes in endocrine cells after vagotomy might be small and dynamic in nature, sensitivity and timing of measurements may also be important factors contributing to the controversies.

The present study was, therefore, performed to detect the changes of antral endocrine cells in mouse after unilateral cervical vagotomy. As the antrum receives its vagal innervation via the anterior vagal trunk that contains nerve fibers from both the left and right vagus, the endocrine cells were examined in the left and right vagotomized animals respectively to establish whether there was a difference in the influence of left and right vagus nerve on the antral endocrine cells.

### Materials and methods

#### Animals

Fifty-four male mice (Bom: NMRI strain, B/S Bomholtgård Breeding and Research Centre, Denmark) aged 3 months, and with an average body weight of 30g were used. The mice were housed in cages, 6 mice in each cage, in a room with artificial light from 6 am to 6 pm. They were fed with a standard pellet diet (Astra-Ewos AB, Södertälje, Sweden) and water ad libitum.

### Unilateral vagotomy and antral endocrine cells

**Table 1.** Number of the animals in vagotomy and sham operation groups.

	SHAM OPERATION	LEFT VAGOTOMY	RIGHT VAGOTOMY
2 weeks	5	6	6
4 weeks	8	6	6*
8 weeks	5	6	6*

\* One mouse died before the end of the experiment.

The mice were kept in the laboratory for 2 weeks before they were subjected to surgery. The mice were randomly divided into three groups, containing 18 mice each. The first group was subjected to left cervical vagotomy, the second to right cervical vagotomy, and the third was sham operated and served as control. Both control and experimental groups were further randomly allocated to three subgroups and killed after overnight fast in a CO<sub>2</sub>-chamber 2, 4 and 8 weeks after the operation, respectively (Table 1). The investigation was approved by the local committee on animal ethics, Umeå University.

#### Vagotomy

The animals were anaesthetised with a mixture of 1 ml midazolam (Dormicum, Roche, Switzerland), 1 ml fentanyl/ fluanison (Hypnorm, Janssen, the Netherlands) and 2 ml distilled water. They were injected with 0.03 ml of this mixture intraperitoneally. Left or right cervical vagotomy was performed by a small skin incision on the neck and the left or the right vagus nerve was identified under a binocular microscope with magnification x10, in close contact with the carotid artery. A minimum of 5 mm of the nerve was resected and prepared for light microscopic identification. The incision was closed with skin sutures. The sham operation group was treated in the same way except that the vagus nerve was left intact.

#### Histological and immunohistochemical techniques

The removed part of the vagus nerve was fixed in 4% phosphate buffered formaldehyde overnight, embedded in paraffin and cut at 5 µm. The sections were stained with haematoxylin-eosin. After sacrificing the animals, the antral part of the stomach was immediately removed, fixed in 4% phosphate buffered formaldehyde overnight and embedded in paraffin. Sections were cut from the specimens at 5 µm and processed for immunohistochemical staining. The primary antisera used were raised in rabbit against chromogranin AB (Euro-Diagnostica, Malmö, Sweden, code no. R 2716-B7), diluted 1:100; gastrin (Euro-Diagnostica, Malmö, Sweden, code no. R783511-B5), diluted 1:2500; serotonin (Euro-Diagnostica, Malmö, Sweden, code no. R871204-B4), diluted 1:1000; and somatostatin (Dakopatts, Glostrup, Denmark, code no. A566), diluted 1:3000. The site of antigen-antibody reaction was

visualised by the avidin-biotin-complex (ABC) method as described previously (El-Salhy et al., 1993). Briefly, endogenous peroxidase activity was inhibited by 0.01% hydrogen peroxide in Tris-HCl buffer (pH 7.6) and the non-specific binding sites were blocked by 1% bovine serum albumin. Sections were treated with the primary antibodies overnight at room temperature, and then incubated with the secondary antibody (biotinylated swine anti-rabbit IgG, Dakopatts, Glostrup, Denmark, diluted 1:200) and the avidin-biotin-peroxidase complex (ABC, Dakopatts, Glostrup, Denmark, diluted 1:200) for 30 min sequentially. They were developed in diaminobenzidine tetrahydrochloride (DAB) followed by slight counter staining in haematoxylin.

Specificity controls included the substitution of the primary antisera with non-immune rabbit serum, pre-incubation of the primary antisera at 4 °C for 24h with the corresponding antigens (50-75 µg/ml diluted antisera), and the replacement of the secondary antibody by non-immune rabbit serum.

#### Computerised Image Analysis

##### Endocrine cells

Quantimet 500 MC image processing and analysis system (Leica, Cambridge, England), linked to a microscope (BX50, Olympus, Japan) was used. The programs used in this system were QWIN (Leica's Windows-based image analysis tool kit) and QUIPS (an interactive programming system). To quantitate the number of endocrine cells per cubic millimeter epithelial cells, an automated standard analysis sequence was used as described in detail previously (El-Salhy et al., 1997). In brief, the nucleated endocrine cells were counted using manual field measurements and the area of epithelial cells was measured using threshold setting. Measurements were performed with a x20 objective and in a frame representing an area of 0.034 mm<sup>2</sup> of tissue. For each type of endocrine cells from each specimen, twenty-five fields randomly chosen from 3 sections (80 µm apart from each other) were used.

##### Epithelium

The areas of the epithelium in both sham operated and vagotomized animals were determined by the same image analysis system using the interactive image editor and erasing the outside region. A 950x950 µm<sup>2</sup> frame and a x4 objective were used. Two fields chosen from three sections cut perpendicular to the mucosa from each mouse were evaluated. The square area of epithelium corresponding to 1 mm baseline of mucosa was calculated.

#### Statistical Analysis

Non-parametric Mann-Whitney test was used for the comparison between the control and experimental

*Unilateral vagotomy and antral endocrine cells*

groups. A p-value <0.05 was considered statistically significant.

## Results

### *Histopathological and immunohistochemical examinations*

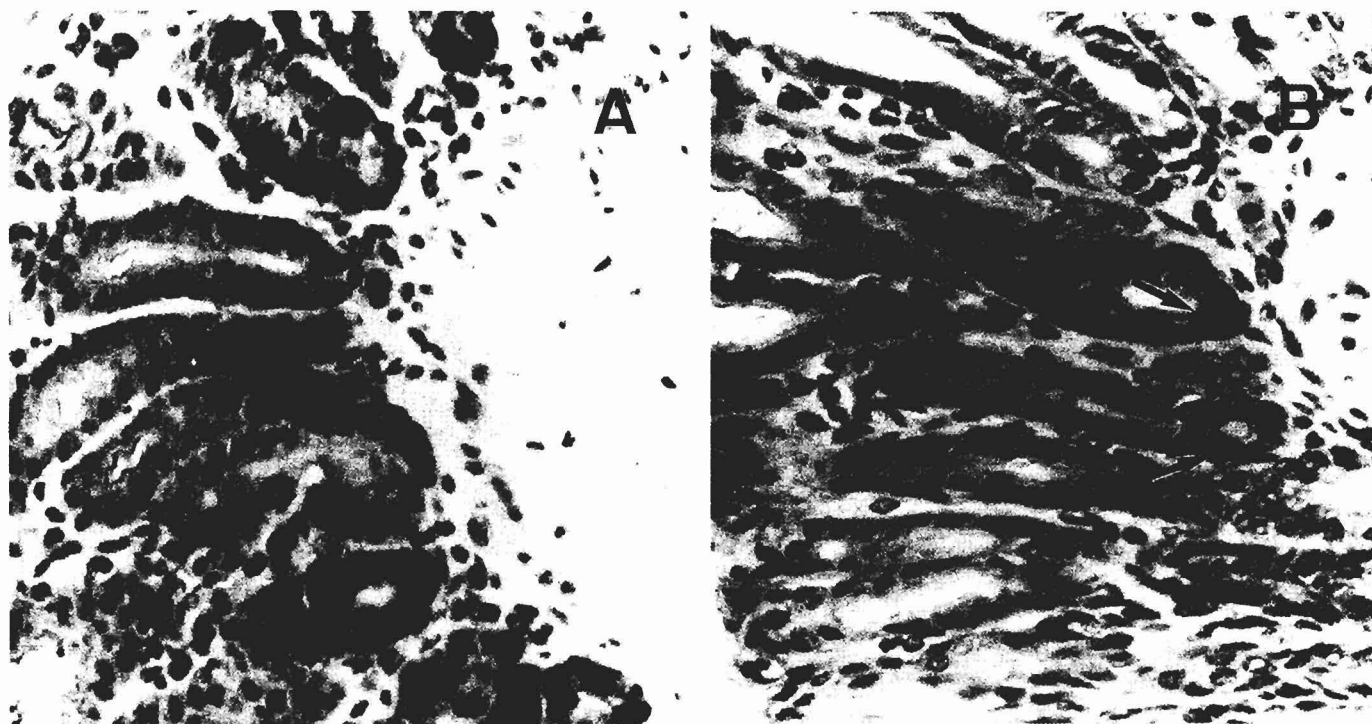
Histological examination confirmed the macroscopically identified and resected vagus nerve. Endocrine cells were localised in the gastric glands and were flask-shaped. Immunoreactive granules were seen

around the nucleus, and at the basal portion of the cells opposite to the luminal pole. In both sham operated and unilaterally vagotomized mice, chromogranin AB-, gastrin-, serotonin-, and somatostatin-immunoreactive cells could be identified. In the vagotomized animals, some of the endocrine cells lost their typical shape. They were oval-shaped, with different degrees of vacuolated cytoplasm and pyknotic nuclei. These morphological changes were mostly found in chromogranin AB-, gastrin-, and serotonin-immunoreactive cells. The most marked alterations were observed after left vagotomy (Fig. 1). The morphological changes increased with the

**Table 2.** The number of various endocrine cells in 1 mm<sup>3</sup> epithelial cells in the antrum of mice after unilateral vagotomy and sham operation

		Cg AB	GASTRIN	SEROTONIN	SOMATOSTATIN
2 weeks	S	15478±3770	23520±6011	3088±874	7966±999
	L	21133±1878	25417±6069	2080±758	11020±1176
	R	15858±2990	32600±3721	4932±592	6815±1426
4 weeks	S	24738±3081	23339±5542	3039±564	5045±871
	L	7746±2100**	10567±5332	3529±1398	2309±637*
	R	6942±1732**	10764±6554	2569±1195	4184±1016
8 weeks	S	30600±6053	26360±831	8202±2020	7086±1458
	L	9385±2216**	12790±1724**	11317±2143	5032±683
	R	10046±1256**	20632±8216	6428±2393	5212±698

Cg AB: chromogranin AB; S: sham operation; L: left vagotomy; R: right vagotomy. Values are mean±SE. \*, p<0.05; \*\*, p<0.01: vagotomy versus sham operation.



**Fig. 1.** Gastrin-immunoreactive cells in the antrum of a control mouse (A) and of a mouse 8 weeks after left cervical vagotomy (B). The arrows point to the gastrin-immunoreactive cells with vacuolated cytoplasm and pyknotic nuclei. Note that the amount of gastrin-immunoreactive cells was reduced in the vagotomized mouse as compared to the control one. x 400

time after vagotomy during the experiment. No immunostaining was seen in any of the negative specificity controls.

#### Computerised image analysis

##### Endocrine cells

The numbers of various antral endocrine cells in both unilaterally vagotomized and sham operated mice are summarised in Table 2.

The number of chromogranin AB-immunoreactive cells was significantly decreased in the mice after either left or right vagotomy as compared to the controls, at both 4 ( $p=0.0013$ , and  $0.0016$ , respectively) and 8 weeks ( $p=0.0043$ , and  $0.0079$ , respectively).

In the animals subjected to left vagotomy, gastrin-immunoreactive cells were decreased though not statistically significantly at 4 weeks after the operation, whereas at 8 weeks (Fig. 1) this decrease became statistically significant ( $p=0.0043$ ). There was no significant change in the right vagotomized mice at any time of the observation period.

The number of serotonin-immunoreactive cells was not influenced by vagotomy. There was no difference between the unilaterally vagotomized and the sham operated mice during the course of the experiment.

Somatostatin-immunoreactive cells were significantly decreased in the mice at 4 weeks after left vagotomy as compared to the controls ( $p=0.043$ ). They returned to the control level at 8 weeks after the operation. However, no such changes were found in the right vagotomized animals.

##### Epithelium

The epithelial areas of antrum in the unilaterally vagotomized and the sham operated mice are summarised in Table 3. No statistically significant difference was found between the groups ( $p>0.2$ ).

#### Discussion

The present study shows that both left and right cervical vagotomy affected the antral endocrine cells in mouse. Left vagotomy seemed to have a more marked influence on the antral endocrine cells than right vagotomy. This is not surprising, as the antral parasympathetic innervation is supplied by the branches from the anterior vagal trunk, which contains nerve fibers mostly from the left vagus. The alteration in the antral endocrine cells observed in this study seemed to be dynamic and depended on the denervated branches of vagus nerve and the observation time after the operation. These may explain, at least partially, the contradictory results obtained earlier by different investigators.

Chromogranin was considered to be a common marker for all the endocrine cells (Facer et al., 1985). However, inconsistent results were also obtained on the

**Table 3.** The epithelium area corresponding to 1 mm baseline of mucosa (mean $\pm$ SE) in the mouse antrum after unilateral vagotomy and sham operation

	AREA OF EPITHELIAL CELLS ( $\mu\text{m}^2$ )		
	Sham operation	Left vagotomy	Right vagotomy
2 weeks	256.08 $\pm$ 46.06	248.53 $\pm$ 40.48	194.51 $\pm$ 20.25
4 weeks	293.46 $\pm$ 46.14	362.23 $\pm$ 69.29	297.54 $\pm$ 24.94
8 weeks	238.72 $\pm$ 9.83	287.51 $\pm$ 33.35	290.13 $\pm$ 38.30

gut endocrine cells by other investigators (Ceting et al., 1989). They showed that the peptide-containing endocrine cells with chromogranin-immunoreactivity varied among species, among gastrointestinal segments and even among members of the same cell population (Ceting et al., 1989). This might explain why the chromogranin AB-immunoreactive cells in some groups did not reflect the sum of various endocrine cell types. The number of chromogranin AB-immunoreactive cells was decreased after 4 weeks and remained at the same level after 8 weeks, post-operatively. This occurred both after left and after right cervical vagotomy. The decrease in chromogranin AB-immunoreactive cells seemed to be caused by cell injury and cell death, as hallmarks of cell death were found at 4 and 8 weeks after vagotomy.

While previous studies agreed that truncal vagotomy or selective proximal vagotomy resulted in hypergastrinemia, the effect of vagotomy on gastrin-immunoreactive cells was controversial (Arnold et al., 1982; Magallanes et al., 1982; Pederson et al., 1984; Holle et al., 1985a,b; Mulholland et al., 1985; Inman et al., 1990; Koop et al., 1993). An increase in gastrin-immunoreactive cells has been reported after vagotomy in rats (Magallanes et al., 1982; Mulholland et al., 1985) and humans (Arnold et al., 1982; Holle et al., 1985a,b; Koop et al., 1993), whereas no change in the number of these cells has been observed in rats (Pederson et al., 1984) and dogs (Inman et al., 1990). In these experiments, vagal denervation was bilateral, or involved anterior and/or posterior vagal trunks which contain the rami from both left and right vagus nerve. The results from the present study indicated that the right vagus nerve had little influence on the gastrin-immunoreactive cells, as no significant effect of right vagotomy on these cells was found. Cell injury and cell death could also explain the decreased number of gastrin-immunoreactive cells observed at 8 weeks after left vagotomy.

The present finding, that neither left nor right vagotomy affected the antral serotonin-immunoreactive cells confirmed the results of earlier studies on vagotomized rats (Portela-Gomes, 1982; Håkanson et al., 1984). It disagreed, however, with Drapanas and co-workers (Drapanas et al., 1971) who found that the serotonin-producing cells almost disappeared 5 to 12 days after truncal vagotomy. An increased amount of tissue serotonin-staining was also reported by other investigations (Tobe et al., 1976; Izumikawa, 1980). However, the experimental period for these studies was



less than 3 weeks. Furthermore, the latter studies did not include a sham operated group of mice as control. It is difficult, therefore, to interpret the results, as the effects of operation and/or anaesthesia per se might influence serotonin-producing cells (Portela-Gomes et al., 1985). Actually, it was noted in the present study, that the antral serotonin-immunoreactive cells did have a tendency to decrease in the mice 2 and 4 weeks after sham operation as compared to those 8 weeks after the surgery.

The right vagotomy seemed to have no impact on the somatostatin-immunoreactive cells. On the other hand, a transient decrease in these cells was observed at 4 weeks after left vagotomy. This result was in agreement with earlier studies on humans with proximal selective vagotomy (Arnold et al., 1982; Koop et al., 1993), but not with those performed on bilaterally truncally vagotomized rats, where the number of somatostatin-immunoreactive cells was increased (Mulholland et al., 1985). Somatostatin inhibits the secretion of gastrin and other peptides by a paracrine mode of action (Schubert and Makhlof, 1991). The alterations in somatostatin-immunoreactive cells should be considered, therefore, in connection with the hypergastrinemia seen after vagotomy.

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

## References

- Arnold R., Hülist M.V., Neuhofer 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.
- Ceting Y., Müller-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.
- Drapanas T., Woolverton W.C., Reeder J.W., Reed R.L. and Weichert R.F. (1971). Experiences with surgical management of acute gastric mucosal hemorrhage: A unified concept in the pathophysiology. *Ann. Surg.* 173, 628-639.
- 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., Sandström O., Näsström E., Mustajbasic M. and Zachrisson S. (1997). Application of computer image analysis in endocrine cell quantification. *Histochem. J.* 29, 1-8.
- 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.
- 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.
- 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.
- Izumikawa F. (1980). Release mechanisms of 5-HT from the gastrointestinal tract in rats. *Arch. Jap. 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.
- 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.
- Mulholland M.W., Bonsack M. and Delaney J.P. (1985). Proliferation of gastric endocrine cells after vagotomy in the rat. *Endocrinology* 117, 1578-1584.
- 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.
- Schubert M.L. and Makhlof G.M. (1991). The somatostatin cell as a local modulator of gastric function. In: *The stomach as an endocrine organ*. 1st ed. Vol 15. Håkanson R. and Sundler F. (eds). Amsterdam. Elsevier. pp 99-110.
- 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). Amsterdam. Elsevier. pp 371-380.

Accepted November 16, 1998