Effects of truncal or highly selective vagotomy on the electron microscopic feature of the rabbit pancreas

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Abstract

Purpose: To perform a comparative study of two types of vagotomy: trunctal vagotomy (TV) and highly selective vagotomy (HSV) effects on pancreatic morphology assessed with light microscope and ultrastructural changes assessed with electron microscope in rabbits 30, 90 and 180 days after the vagotomy.

Material and methods: The experiments were carried out on 89 male Popielno white rabbits, aged 4-6 months, 2.5 to 3.0 kg of body weight. On the 30th, 90th and 180th day after the vagotomy, rabbits were sacrificed and the pancreases were taken for the light and electron microscopic evaluation.

Results: The regressive changes coexisting with adaptive like renewal of epithelial cells and mild interstitial fibrosis resulting from vagotomy were more pronounced in the early post-operative period and tended to normalize in the later post-operative one. All the changes seen in post-operative period were more prominent after TV than after HSV.

Conclusions: Both truncal and highly selective vagotomy affects evidently the morphology and ultrastructure of the pancreas in rabbits however the changes after the first procedure were more advanced than after the latter one. The intensity of the changes is highest early after these operations and tend to normalize in the later post-operative period.

Key words: highly selective vagotomy, truncal vagotomy, pancreas morphology, pancreas ultrastructure, rabbit.

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Introduction

The vagal cholinergic pathways play an important role in neurohormonal regulation of pancreatic function. The morphological changes of the pancreas, as a result of vagotomy, for surgical treatment of peptic ulcer have not been adequately investigated.

A decreased size of pancreatic acini and the edema of beta islet cells occurring within the first weeks following total vagotomy with pylorotomy in rats have been described [1]. Lorenz et al. [2] observed a decreased size of pancreatic acini and increased number of alpha cells of the islets 5 weeks after total vagotomy, whereas 3 weeks after operation their number may be significantly reduced in rats.

According to Staszyc and Królikowska-Prasał [3], vagotomy adversely affects the metabolic processes in the pancreas, which manifests with abnormal enzymatic activity. Islet cells were found to regenerate earlier than acinar cells, as it was supported by histochemical examination. Moreover, during the early post-operative period the truncal vagotomy was found to influence the neurogenic secretion. Following truncal vagotomy, the number of zymogen granules was initially decreasing, but subsequently increased in the rats.

Our own experiments performed in dogs, evaluating the morphological and histochemical changes in the pancreas resulting from different types of vagotomy: truncal, and highly selective, showed that the degree of the abnormalities was highest during the early post-operative period. In later periods, the severity of morphological and histochemical changes decreased. The most significant structural abnormalities were observed after truncal vagotomy [4].

In the available literature we could find neither an ultrastructural evaluation of the pancreas following different types of vagotomy, nor the assessment of its influence on the endocrine and exocrine function of this organ. The observations of the neurological changes in the pancreas, as a result of vagotomy, are not consistent. Radke and Stach [5-7] did not find any ultrastructural changes in the pancreatic neurons, which

Table 1. **Experimental and control groups of rabbits**

Type of vagotomy	Duration of the experiment (days)		
	30	90	180
	Number of animals		
Highly selective vagotomy	11	13	11
Total vagotomy	7	12	10
Control I (sham operation)	3	3	3
Control II (no operation)	7	5	4

is inconsistent with the findings of Shashirina [8]. The trophic effect of truncal vagotomy on the rat pancreas, manifesting itself by an increased weight of the organ, was described by Koop et al. [9], Tiscornia et al. [10] and Büchler at al. [11].

The gastrointestinal complications of vagotomy, known from the clinical practice, may result from total or partial parasympathetic denervation. Most of gastrointestinal complications are well known to develop directly after the surgery, and to be of transient nature [3,12-14]. Evaluation of such complications in humans is difficult due to the lack of possibility of morphological, histochemical and ultrastructural assessment in the postoperative period. Therefore, reasonable animal experiments could be helpful to explain the pancreatic complications of the vagotomy. The results of such experiments should be applied with caution in the clinical practice, however, they could bring some clinical implications, allowing the appropriate therapeutic or prophylactic intervention.

Considering the different extent and selectivity of parasympathetic denervation of the stomach and as the consequence of the pancreas after different types of vagotomy the morphological alterations of the pancreas could be less or more advanced

Therefore the aim of the study was to perform a comparative assessment of the influence of different types of vagotomy: truncal vagotomy (TV) and highly selective vagotomy (HSV) on the morphology and ultrastructure of the rabbit pancreas after 30, 90 and 180 days following truncal vagotomy and highly selective vagotomy.

Material and methods

The experiments were carried out on 89 male Popielno white rabbits, aged 4-6 months, with body weight varying from 2.5 to 3.0 kg. The animals were housed in standard laboratory conditions (room temperature, daily light, natural food and tap water ad libitum). The care was provided according to current guidelines for the use of laboratory animals and experiment was performed according to the updated Helsinki Declaration. The study received an approval of local bioethics commission. The animals were allocated to 2 experimental and 2 control groups, depending on the type of vagotomy (*Tab. 1*).

The rabbits were fasted overnight before experiment with free access to tap water. Atropine sulphate was administered subcutaneously in the dose of 0.1 mg per kilogram of body weight. The general anaesthesia was induced by an open-method inhalation with purified ethyl ether. Central superior laparotomy was then performed. In the animals from the control group I (sham operation) laparotomy was performed and a loose catgut ligature was placed on the trunk of the vagus nerve, while no surgery was performed in the control group II.

Total vagotomy was performed by cutting the vagus nerve approximately 0.5 cm below the diaphragm. To perform the highly selective vagotomy after dissection and ligation of blood vessels, the laminae of the lesser omentum were cut approximately 0.5 cm to the lesser curvature of the stomach, with a cut leading from the oesophagus towards the pylorus, reaching the border between the body and the pyloric cavity. Next the small branches leading from the lesser curvature of the stomach to the anterior and posterior groups of the branches of the vagus nerve were cut. This technique saved the branches supplying the celiac plexus, the liver and the pancreas. The abdominal cavity was then closed with two layers of dexon “0” interrupted noose sutures.

The animals were sacrificed on day 30th, day 90th or day 180th after the surgery, and immediate laparotomy was performed with dissection of the pancreas for morphological examination. The weight of the organ was also examined.

Light microscopy

The specimens for morphological examination in the light microscopy were taken from the left and the right portion of each pancreas. The material was fixed for 15 days in a 10% neutral formaldehyde solution; then dehydrated and embedded in the paraffin. The paraffin slices were stained with hematoxylin and eosin (H&E)

Electron microscopy

Following the truncal and highly selective vagotomy in rabbits, specimens for electron microscopy were collected from two random animals in each experimental group (a total of 18 experimental + 4 control specimens).

Small specimens of the rabbit pancreases were stabilized at room temperature, in a 4% glutaraldehyde solution in 0.1 M cacodylate buffer at pH=7.4, for six hours. Scraps were then rinsed for 12 hours in 0.1 M cacodylate buffer at pH=7.4; the buffer solution was changed three times during this process. Subsequently, the preparations were additionally stabilized in a 1% OsO_4 water solution in 0.1 M cacodylate buffer at pH=7.4, for two hours, at the temperature of 4°C. The preparations were then dehydrated in a series of ethanol solutions of increasing concentration (30%, 50%, 70%, 90%, 96%, 99.8%), a mixture of propylene oxide and Spurr resin, a series of volume proportions 1:2, 1:1, 2:1, and twice in pure propylene oxide. Preparations were then covered with Spurr Low Viscosity resin.

Contrast-enhancement of ultra-thin preparations in 8% uranyl acetate solution for 45 minutes and then for ten minutes in plumbum citrate solution, according to Raynolds, was applied. Semi-thin slices, 0.75 μm, were stained in a 1% methylene blue with 1% Azur II solution in 1% water solution of borax. The preparations were evaluated and photographic documentation was made using a TESLA BS-500 electron microscope.

Statistical analysis

The results are descriptive, therefore the statistical analysis is not applicable.

Figure 1. **EM – in acinar cells of the rabbit pancreas, 30 days after TV, we can notice slight widening of smooth and rough endosplasmatic reticulum channels, single myelin figures (arrow) and phagosomes (F). Zymogen granules (Z) have normal ultrastructural feature. Ultimate magnification: x18 000**

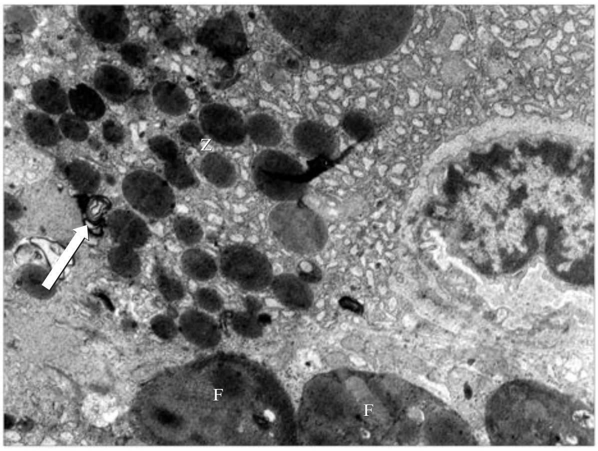
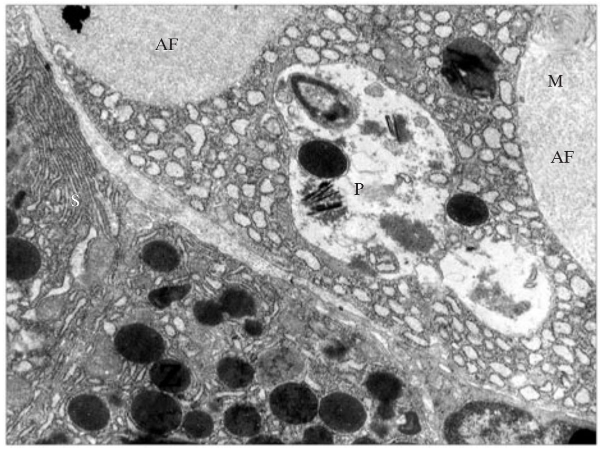


Figure 2. **EM – in one of the cells of the exocrine rabbit pancreas, 90 days after TV, the numerous autophagy vacuoles (AF) containing the material of moderate free-electron density, cell organelles, paracrystalline structures (P) and myelin figures (M) could be seen. In remaining cells there are numerous zymogen granules (Z), and in all cells an abundant rough endosplasmatic reticulum (S) with slight focal broadening of the channels could be noticed. Ultimate magnification: x14 000**



Results

Histological changes

Light microscopy findings comprised foci of dyschylic edema observed in individual rabbits. Another finding was foci of fibrosis within the parenchyma of the pancreas. It needs to be emphasized that – similarly to light microscopic findings – dyschylic edema was found more frequently at earlier post-operative stages. The degree of the phenomenon in the early post-operative period was not very severe. Focal fibrosis of various severity occurred occasionally, similarly to the microscopic findings described above.

Ultrastructure of the rabbit pancreas after the truncal vagotomy (TV)

Thirty days after truncal vagotomy the enlargement of the intercellular spaces, mainly in the exocrine portion of the pancreas, was observed. Within the cytoplasm of the exocrine cells the proliferation and enlargement of tubules of the smooth-surfaced endoplasmic reticulum, mitochondrial edema, numerous myelin figures and autophagic vacuoles were found. Occasionally, myelin figures were found within the enlarged intercellular space, and sometimes within the concavities of the nucleus, filled with cytoplasm. The number and the electron density of zymogen granules varied. Occasionally the degradation resulting in the occurrence of myelin figures in the affected granules could be observed (*Fig. 1*). Besides this ultrastructural features of extensive protein synthesis were observed in numerous cells, including enlargement of the rough-surfaced endoplasmic reticulum tubules, filled with fine fibrous contents and a conspicuous Golgi apparatus. Within the endocrine component of the gland, the ultrastructural findings involved mainly the increased number of granules, observed most frequently in type B cells. Numerous fibroblasts surrounding the blood capillaries were observed, accompanied by fine fibrous matter and collagen fibres.

Ninety days after TV the alterations in the exocrine portion of the pancreas resembled those observed 30 days following the surgery, but were less severe. Large autophagic vacuoles were more frequent, containing cell fragments, crystal-like structures and numerous, well-developed tubules of the rough-surfaced endoplasmic reticulum (*Fig. 2*). In some endocrine cells, mostly type B islet cells, a focal degeneration of the granules was seen. Within the intercellular space, especially in the vicinity of blood vessels, the bundles of collagen fibres, active fibroblasts and macrophages were found. Occasionally, loose cell fragments were detected within the intercellular space, which suggests damage to the cell membranes.

One hundred eighty days after TV a variable, usually big number of zymogen granules in the cells of the exocrine portion of the pancreas and ultrastructural features of extensive protein synthesis were observed. Some cells contained numerous autophagic vacuoles and only few zymogen granules. Sporadically, signs of colliquative necrosis was observed in the exocrine cells (*Fig. 3*). Increased number of granules was observed in type A, B and D endocrine cells, but especially in B cells the granules were markedly condensed and contracted. These changes were accompanied by a marked mitochondrial edema. However, in most cases, the endocrine cells of the pancreatic islets showed a normal ultrastructural pattern. Intercellular spaces, especially the pericapillary area, often contained active fibroblasts and bundles of collagen fibres.

Ultrastructure of the rabbit pancreas following highly selective vagotomy (HSV)

Thirty days after HSV a variable number of zymogen granules of various electron densities was observed in the cells of the exocrine portion of the pancreas. Enlargement of the

Figure 3. EM – focal colliquative necrosis of pancreatic acinar cells after 180 days from TV. Numerous autophagy vacuoles (AF) are visible. Ultimate magnification: x15 000

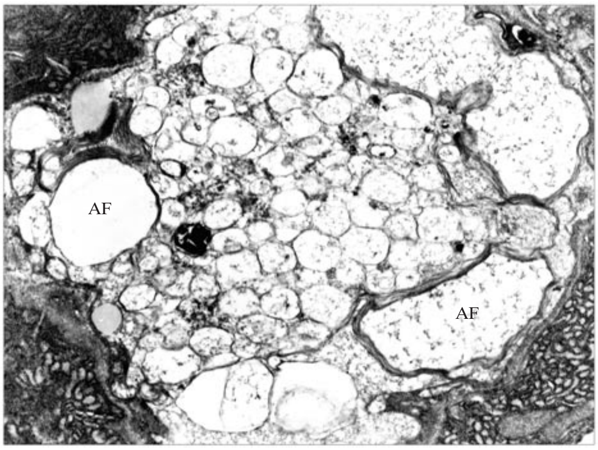


Figure 5. EM – marked increase of the number of zymogen granules 90 days after HSV. Visible swelling of mitochondria (arrows). Ultimate magnification: x14 000

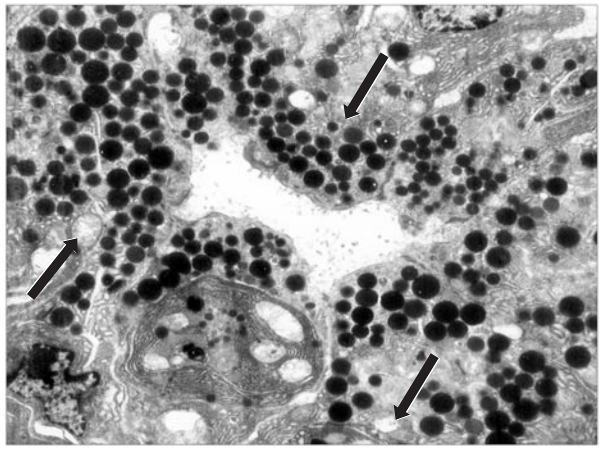
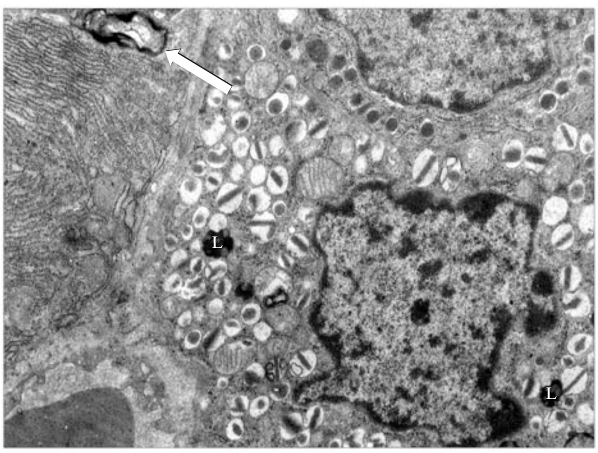


Figure 4. EM – in beta cell of the rabbit pancreas, 30 days after HSV, shrunk endocrine granules and lysosomes (L) can be noticed. In exocrine pancreas there is a myelin figure (arrow) and abundant rough endosplasmatic reticulum. Ultimate magnification: x16 000



rough-surfaced endoplasmic reticulum, filled with fine fibrous contents, was a common finding. Moreover, in the cytoplasm of those cells autophagic vacuoles were observed, containing membranous convolutions resembling myelin figures, fragments of organelle and fine granular material. In the endocrine cells, especially type B cells, condensed and contracted granules as well as individual myelin figures were observed (*Fig. 4*). No fibrosis was detected in the evaluated specimens.

Ninety days after HSV the alterations resembled those described in the previous group, but they were significantly less severe. Autophagic vacuoles were occasionally seen in the cytoplasm of the cells of the exocrine portion of the pancreas. Myelin figures could be also observed in the enlarged intercellular space. Well-developed rough-surfaced endoplasmic reticulum, filled with fine fibrous material was seen in many cells of the exocrine portion of the organ. The number and the electron density of the zymogen granules varied. In certain cells, especially those equipped with granular endoplasmic reticulum, zymogen granules were not seen at all, whereas in other cells the number of such granules was moderate or marked (*Fig. 5*). In the endocrine cells, especially type B cells, condensed and contracted granules as well as myelin figures, secondary lysosomes and autophagic vacuoles were found. Chaotically distributed collagen fibres and fragments of fibroblasts were seen in the intercellular space and the interstitium, especially in the vicinity of blood capillaries.

One hundred eighty days after HSV large autophagic vacuoles and myelin figures were only occasionally observed in the cytoplasm of the exocrine portion cells. Individual exocrine cells containing condensed and fragmented nuclear chromatin as well as dark cytoplasm, which might suggest apoptotic processes, were found. Cells with well-developed rough-surfaced endoplasmic reticulum, containing fine fibrous matter in ergastoplastic cisterns, were a frequent finding. Numerous pores of the nuclear membrane were observed. The number of zymogen granules, their shape and density varied. Exceptionally alpha (glycogen) granules were seen in the cytoplasm of the cells of the endocrine part of the pancreas. Some cells, mostly type B islet cells, contained contracted granules and numerous secondary lysosomes.

Discussion

Vagotomy, a conservative surgical procedure used for the treatment of peptic ulcer, remains the subject of experimental research, aimed at the explanation of the enzymatic and hormonal relations and structural changes within the gastrointestinal tract, especially the pancreas, as possible consequences of the surgery. Disturbances concerning innervation of the pancreas and blood supply to the organ result in trophic changes [9,11]. Radke and Stach [5-7] did not find any ultrastructural changes in axons and organelle of the sympathetic and parasympathetic neurons (vesicles, mitochondria, microtubules) at 14 days and 5 months after truncal vagotomy in dogs. However, Shashirina [8], while evaluating the morphological changes in the neural system of the pancreas of the guinea pig after vagotomy, described chromatolysis and edema in most neurons evaluated 7 days after the surgery and increased number of atrophic cells 14 days after operation. The size of neurons, found to be increased 7 days after surgery, decreased again on day 14th after vagotomy. Similar changes were observed in the nucleus. Sixty days after surgery morphometric indices of neurons were comparable to those observed in the control group. According to this author three post-operative stages can be distinguished for truncal vagotomy:

1. alterations related to disturbed functioning of the cell (7 days after the surgery),
2. destructive changes (approx. 14 days after the surgery),
3. compensatory changes and recovery (60 days after the surgery) [8].

Both Büchler et al. [11] in rats after truncal vagotomy accompanied by pyloroplasty and Koop et al. [9] in rats after truncal vagotomy, observed increased weight of the pancreas. However, they did not find any morphological differences in the exocrine pancreas before and after the operation. The concentrations of DNA and trypsin in homogenized pancreas were increased, but the activity of lipase was found to be decreased in both experiments. Similarly, Tiscornia et al. [10] observed a trophic effect of truncal vagotomy in rats on the exocrine part of the pancreas, accompanied by an increased activity of lipase and unchanged activity of other enzymes. This may prove that the pancreas is controlled by numerous secretory stimuli, which equilibrate abnormal secretion in the early post-operative period. In the early post-operative period (up to 5 months) vagotomy affects also the systems responsible for controlling of the glucose concentration in the blood. The vagus nerve, by stimulating the secretion of insulin, inhibits glycogenolysis.

Koop et al. [9] in their morphometric assessment of the endocrine component of the pancreas of rats after truncal vagotomy, described a relative decrease of the size of this portion in favor of the exocrine portion. The total number of endocrine cells remained unchanged. It suggests a trophic effect of truncal vagotomy on the exocrine, but not on the endocrine portion of the pancreas. In rats, the number of alpha islet cells was found significantly reduced at 3 weeks after truncal vagotomy, while this number was increased at 5 weeks after the surgery. The alpha/beta cell ratio was 1:2 in control animals, 1:5 in experimental animals in 1st week after the surgery, and 1:1 in the 3rd week after the surgery [2]. Truncal vagotomy, apart from its direct effect on the gland cells, affects them by influencing the blood supply, as the pancreatic interstitium – the stroma of the organ – responds to vagotomy with alterations of its enzymatic activity, similarly to the gland tissue.

Available literature indicates on gastrin and the vagus nerve as the two factors bearing most responsibility for the integrity of the mucous membrane of the stomach [15,16]. The function of endocrine cells in the gastrointestinal tract also remains under control of gastrin and the vagus nerve [6,17].

The light and electron microscopy of the peptic cells in patients before and one year after highly selective vagotomy did not show any significant differences within those cells [18].

No ultrastructural alterations were found in the neurons of the submucous ganglions of the intestine of the pig on day 1 after truncal vagotomy. From 3rd day to 5th day after the operation some presynaptic axons of the submucous neurons showed various degree of degeneration. The signs of degeneration involved mostly edema, mitochondrial vacuolization with broken mitochondrial crests, as well as clustering of synaptic vesicles. The 7th day after operation, further degeneration of terminal axons was found. On the 10th day following the surgery the described changes reached their maximal severity, and subsided 30 days after vagotomy. No differences were observed in the number of the axons between day 10th and day 30th after operation. These findings were more frequent as a result of left as compared to right vagotomy.

Huchtebrock et al. [19] observed signs of degeneration of internal pancreatic nerves after truncal vagotomy, resection of the solar and the superior mesenteric ganglions, and a combination of the two procedures. Sixty days after the surgery the hypertrophy of nerve fibers was observed, however, the integrity of the internal pancreatic ganglions remained unaffected. The concentrations of substance P (SP) and neuropeptide Y (NPY) decreased significantly after the denervation, despite the fact that the peptidergic nerves had not been damaged neither by the truncal vagotomy, nor the ganglionectomy, nor the combination of the two procedures. The canine pancreas should be thought to posses peptidergic innervation, independent, except for SP and NPY, of the integrity of the extrapancreatic nerves [19].

Our own studies in dogs did not reveal any significant changes of the pancreas weight as a consequence of truncal and selective vagotomy, compared to control animals [11]. However, in the group of rabbits, 180 days after truncal and highly selective vagotomy, the weight of the organ was significantly increased [20,21]. Our own studies, similarly to other authors [6,11,12], may prove that functional, but not morphological changes of the pancreas are the most significant consequences of vagotomy in the early post-operative period.

The above described electron microscopy findings in rabbits, as well as light microscopy, indicate that:

1. The changes resulting from vagotomy are more pronounced in the early post-operative period.
2. The changes resulting from vagotomy tend to normalize in the later post-operative period.
3. The effects could be mainly of functional nature, including, among others, increased protein synthesis as an electron microscopy finding.
4. Regressive changes coexist with adaptive changes, renewal of epithelial cells and mild interstitial fibrosis; those alterations were seen in all post-operative stages, and were more prominent after TV than after HSV.
5. The evolution of the alterations in the pancreas, evaluated by means of light and electron microscopy, correlates with the neurological complications of vagotomy in the pancreas, described by Shashirina [8].

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