Microscopic study of the rat pancreas after denervation

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SUMMARY

The aim of this study is to demonstrate that the denervation of the pancreas may affect the enteric neuronal plexus, which controls both the endocrine and exocrine parts of the pancreas. By using the light microscope, the histological changes of the islets of Langerhans and the pancreatic acini in the rat pancreas were studied two and three weeks after sympathectomy and truncal vagotomy. Moreover, measurements of the changes in fasting blood glucose levels and glucose tolerance tests in the control and experimental animals were recorded.

Atrophic changes and degeneration of the pancreatic acinar cells and islets of Langerhans cells were observed after both sympathectomy and vagotomy. Biochemical measurements of fasting blood, and the glucose tolerance tests after sympathectomy and vagotomy were increased significantly, which is consistent with the histological results. The results of this study explain that the exocrine and endocrine parts of the pancreas are dependent on both sympathetic and parasympathetic innervation via the enteric plexuses of the rat pancreas. These results establish a firm correlation between the autonomic innervation and the enteric plexus, which controls the function of the endocrine and exocrine parts of the pancreas.

Key words: Rat pancreas – Truncal vagotomy – Sympathectomy – Enteric plexus – Microscopic study

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INTRODUCTION

The pancreas is a unique abdominal organ in that it is classified as both an endocrine and exocrine gland. The innervation of the pancreas is comprised of both an intrinsic component that consists of many intrapancreatic ganglia and an extrinsic component made of neurons lying outside the digestive tract and belonging to the sympathetic and parasympathetic systems (Furness and Costa, 1987; Furness and Bornstein, 1991; Gershon et al., 1994; Furness et al., 1995; Salvioli et al., 2002). Since the pancreas develops as an outgrowth of the gastrointestinal tract during fetal life, it has been proposed that it has ganglia, which is analogous to the ganglia of the enteric nervous system. These ganglionic structures are randomly scattered throughout the pancreatic parenchyma and represent the intrinsic neural component of the pancreatic nerve supply (Tiscornia, 1977; Holst, 1993; Salvioli et al., 2002).

Sympathetic control of the pancreas originates from nerve cell bodies in the hypothalamus; axons from these neurons exit the spinal cord through the ventral roots at the level of all thoracic and upper two lumbar segments of the spinal cord. These spinal nerves form the preganglionic nerve fibers supply either the paravertebral ganglia of the sympathetic chain via communicating rami of the thoracic and lumbar nerves, or the celiac and mesenteric ganglia via the splanchnic nerves. The postganglionic sympathetic fibers exit the ganglia accompanied by the parasympathetic fibers as mixed autonomic nerves that supply the pancreas along the branches of the celiac trunk. It innervates the intrapancreatic ganglia, islets, blood vessels and, to a lesser extent, the ducts and acini. Moreover, preganglionic sympathetic nerve fibers can also

enter the pancreas and synapse directly with the enteric plexus of the pancreas (Ahren et al., 1981; Babic and Travagli, 2016).

On the other hand, the parasympathetic nerve fibers take origin from the dorsal vagal nucleus, and some may originate from the nucleus ambiguous of the brain stem. These fibers run through the vagus nerve; most of those supplying the pancreas run within the posterior vagal trunk near the lesser curvature of the stomach and the pyloric region to synapse in the myenteric plexus. The postganglionic fibers supply the pancreas directly and indirectly through the enteric plexus of the pancreas (Ahren, 2000; Babic and Travagli, 2016).

It is reported that vagal impulses act on the exocrine pancreas via pancreatic ganglia of the enteric plexus, where the impulses are carried via postganglionic fibers to the acinar cells stimulating the secretion of the pancreatic enzymes (Brissova et al., 2005; Love et al., 2007; Babic and Travagli, 2016). However, a study done by Gershon et al. (1994) suggests that the intrinsic neurons of the enteric plexus can influence the function of the exocrine and endocrine parts of the pancreas. On the other hand, sympathetic nerves may regulate hormone secretion in human islets by controlling local blood flow or by acting directly on the islet cells (Gershon et al., 1994; Rodriguez-Díaz et al., 2011).

Recently, several important studies have been concentrated on pancreatic islets transplantation (Hirshberg et al., 2003), as well as pancreatic denervation and its complications (Barzilai et al., 1987). Therefore, the aim of this study is to investigate the role of the enteric plexuses on the exocrine and endocrine portion of the rat pancreas after denervation, by observing the histological changes of the endocrine islets and the exocrine pancreatic acini, and also by measuring the changes in glucose concentration before and after denervation, so we can establish a firm correlation between the autonomic denervation of the pancreas and the complications encountered in different clinical aspects related to the pancreas, such as the failure of the pancreatic transplantation, and the diabetic symptoms of patients who suffered from an episode of neurogenic shock, and to know if these complications are mediated by the effects of the enteric plexus of the pancreas.

MATERIALS AND METHODS

In this study, twenty-four adult albino rats of both sexes were used. The rats' body weight ranged from 250-500 gm. All animals were treated according to the National Institution of Health guideline for experimental animals. Two separate experiments were done; the first was truncal vagotomy, while the second was sympathectomy. Each one was done on twelve rats which were randomly selected.

Truncal vagotomy was performed by cutting the anterior and posterior vagal trunks around the esophagus below the diaphragm, each trunk was isolated, and then a piece of 1 cm was cut and removed between two ligatures. On the other hand, the sympathectomy was performed by cutting the splanchnic nerve fibers projecting to the pancreas, which means cutting all nerve fibers around the celiac trunk and its branches, and the nerve fibers around the renal arteries. The rats were anaesthetized by intraperitoneal injections of sodium pentobarbital (25 mg/kg body weight).

The twelve rats in each experiment were divided into three subgroups. The first subgroup of 4 animals was the control group, the second subgroup was allowed to survive for two weeks after the surgery, while the third subgroup was allowed to survive for three weeks after the surgery.
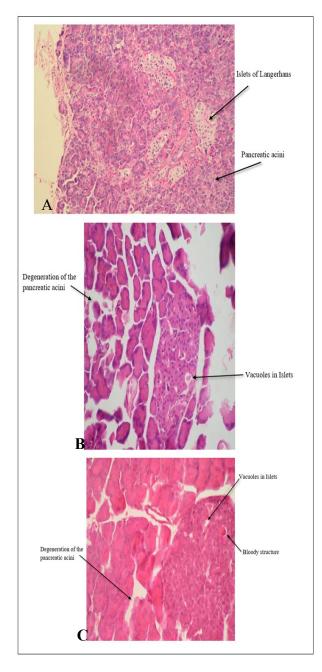
Measurements of the fasting blood glucose levels for the rats at different intervals of time after the surgery were done, in addition to a glucose tolerance test measured two hours after intravenous injection of 0.5 gm/kg body weight of glucose.

All animals were sacrificed by the perfusion method through the left ventricle of the heart, first by normal saline, then with 10% formaldehyde fixative. After their death, the pancreas was dissected out, cut into small pieces and put in fresh fixative for 48 hours. Tissues were then dehydrated in alcohol, cleared in xylene and then embedded in paraffin wax. Tissue blocks were cut into 10mm thick section, then rehydrated and stained with hematoxylin and eosin. The tissues were then examined with the light microscope.

RESULTS

Microscopic examination of the pancreas of the control group showed that the exocrine portion comprises the bulk of the organ and is subdivided into lobes and lobules by connective tissue septa. Each lobule consists of closely packed secretory acini. Each acinus is composed of several pyramidal shaped cells possessing a round nucleus. However, the endocrine portion of the pancreas is composed of small spherical clumps of different types of cells - islets of Langerhans - which is richly supplied by capillaries. The islets of Langerhans are haphazardly scattered among the exocrine acini of the pancreas (Fig. 1A).
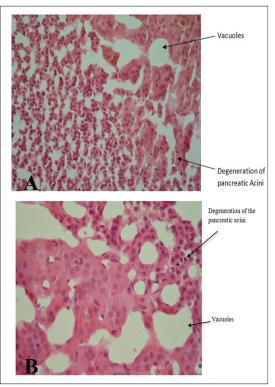
Measurements of the fasting blood glucose levels and the glucose tolerance test of the control group were normal (Table 1 and 2). Two weeks after the truncal vagotomy, the microscopic examination of the exocrine portion of the pancreas showed numerous large vacuoles between the secretory acinar cells due to degeneration, the pancreatic acinar cells showed shrinkage, the nuclei were dentate and the cells were not bound by clear cell membrane (Fig. 1B). Three weeks after the truncal vagotomy, more structural changes and

Fig 1. Light micrograph of sections from rat pancreas. (**A**) Control rat, showing the islets of Langerhans and the pancreatic acini. (**B**) Rat pancreas two weeks following truncal vagotomy, showing degenerative changes in the islets of Langerhans cells, it also shows the formation of the large vacuoles between the pancreatic serous acini due to degeneration. (**C**) Rat pancreas three weeks following truncal vagotomy, showing the enlargement of the vacuoles in the pancreatic acini and the further degenerative changes in the islet cells and a bloody structure. (H&E staining; A-B: x200, C: x400).

Fig 2. Light micrograph of sections from rat pancreas. (**A**) Rat pancreas two weeks following sympathectomy, showing degenerative changes in the islets of Langerhans cells, it also shows the formation of the large vacuoles between the pancreatic serous acini due to degeneration. (**B**) Rat pancreas three weeks following sympathectomy, showing the enlargement of the vacuoles in the pancreatic acini and the further degenerative changes in the islet cells. (H&E staining; A: x200, B: x400).

Table 1. Fasting Blood Glucose levels in mmol/L for the control group, after truncal vagotomy and sympathectomy at different time intervals

	Control	2 weeks	3 weeks
After Truncal	3.3-4.4 mmol/	6.3-8.5 mmol/	8.5-10.2
After Sympa-	3.5-4.5 mmol/	6.5-8.8 mmol/	8.7-10.5

Table 2. Glucose Tolerance Test levels in mmol/L for the control group, and after truncal vagotomy and sympathectomy at different time intervals

	Control	2 weeks	3 weeks
After Truncal Vagotomy	4.2-4.9 mmol/L	8.9-11.2 mmol/L	10.4-14.2 mmol/L
After Sympathectomy	4.0-4.5 mmol/L	8.4-10.6 mmol/L	8.9-12.6 mmol/L

more vacuoles were observed due to progressive degeneration (Fig. 1C).

Microscopic examination of the islets of Langerhans two weeks after truncal vagotomy showed degenerative and nuclear structural changes such as dentate nuclei (Fig. 1B). Additional degeneration was observed in the islet cells three weeks after truncal vagotomy as some of the cells had disappeared completely and were replaced by connective tissue and bloody structures which could be due to rupture of capillaries (Fig. 1C).

Measurements of the fasting blood glucose levels at different intervals of time after truncal vagotomy showed a significant increase in the glucose

levels in comparison to the control group (Table 1). Moreover, the glucose tolerance test two hours after the injection of glucose in the animals who survived two and three weeks after truncal vagotomy was also significantly increased (Table 2).

Two weeks after sympathectomy (published results), microscopic examination of the exocrine portion of the pancreas showed degenerative changes represented by the development of large vacuoles between the secretory acinar cells due to shrinkage, and the acini were not bounded by a clear cell membrane (Fig. 2A). After three weeks more structural changes were observed, as much more vacuoles were noticed, and some cells appeared without nuclei and indistinctive cellular membranes (Fig. 2B).

Microscopic examination of the endocrine portion of the pancreas two weeks after sympathectomy (published results) showed degenerative changes of the cells. Changes in their nuclear structure were observed such as dentate nuclei (Fig. 2A). Moreover, degenerations were observed in the islet's cells three weeks after sympathectomy, as some of the cells had disappeared completely and were replaced by connective tissue and bloody structure (Fig. 2B).

Measurements of the fasting blood glucose levels at different intervals of time after sympathectomy (published results) showed a significant increase in glucose levels in comparison to the control group (Table 1).

Moreover, the glucose tolerance test two hours after the injection of glucose in the animals who survived two and three weeks after sympathectomy (published results) were also significantly increased (Table 2).

DISCUSSION

In this study a correlation between the autonomic innervation and the histological changes and metabolic abnormalities of rat pancreas has been demonstrated.

It is reported that the vagal innervation of the pancreas has direct effect on the exocrine acinar secretions, as vagal impulses stimulate the secretion of the different pancreatic enzymes (Brissova et al., 2005; Love et al., 2007; Babic and Travagli, 2016). These results agree with our microscopic findings, as the loss of innervation after the truncal vagotomy showed a high degree of atrophy and degeneration of the serous acinar cells.

Moreover, several studies have revealed that truncal vagotomy leads to a substantial decrease in the pancreatic exocrine digestive enzyme secretions due to parasympathetic denervation and the interference with the pancreatic enteric reflexes (Thambugala and Baron, 1971; Wormley, 1972; Malagelada et al., 1974). Highly selective vagotomy, on the other hand, does not impair the exocrine function of the pancreas that much, but can produce a satisfactory decrease in the gastric acid secretion; thus, it is proposed to use the highly selective vagotomy as an alternative treatment for duodenal ulcer (Cox and Bond, 1964; Fraser et al., 1983; Rodríguez-Díaz et al., 2011).

The microscopic findings of the islet cells of the rat pancreas after vagotomy have also shown atrophy and subcellular changes. Furthermore, there was a substantial increase in the glucose levels in the blood after the truncal vagotomy. All these results suggest that the vagal innervation of the pancreas have a direct effect on the endocrine function of the pancreas. Our conclusion was demonstrated in other studies: in a study conducted by Rodríguez-Diaz et al. (2011), they found that the human islets are sparsely contacted by autonomic fibers, few vagal fibers directly penetrate the islet itself suggesting a direct innervation. According to Rossi et al, the parasympathetic fibers that innervate the endocrine part originate from intrapancreatic ganglia, which are connected with the fibers from the vagus nerve (Rossi et al., 2005). In a study conducted by Nishi et al. (1987) using electrical vagal stimulation, the isolated perfused rat pancreatic islets (in vivo techniques) produced an increase in both insulin and glucagon secretion in proportion to the pulse frequency. When atropine was infused, both insulin and glucagon responses to vagal stimulation were partially suppressed. Previous studies have demonstrated that the vagus nerve also contains adrenergic fibers (Petterson et al., 2009). It might be possible that adrenergic fibers in the vagus nerve contribute to insulin and glucagon secretions induced by vagal stimulation; yet in the study by Nishi et al. (1987) the insulin and glucagon responses have not changed after the administration of certain alpha receptor antagonists; thus, it seems unlikely that the adrenergic fibers play an important role in the regulation of the vagally induced pancreatic hormone secretion (Franco-Colin et al., 2006).

Therefore, the effect of truncal vagotomy on the exocrine and endocrine parts of the rat pancreas was demonstrated in this study and it came in agreement with literature.

The sympathetic effect on the endocrine part of the pancreas in this study was very similar to that of the previous study conducted by Al-Muhtaseb et al. (2013). Moreover, the correlation between the sympathetic innervation and metabolic abnormalities of the pancreas has been demonstrated in previous studies using different methods (Kvist et al., 2002; Franco-Colin et al., 2006). All of which confirms our results.

In a study conducted by Ahren et al. (1987) regarding the autonomic regulation of the pancreatic islet secretions, the electrical stimulation method was used on the sympathetic splanchnic nerves along the pancreatic artery, combined with the chemical blockade of the cholinergic preganglionic fibers: this has provided a satisfactory way to de-

tect the direct effects of the sympathetic nerve stimulations on the pancreatic islets without the parasympathetic effect. After stimulation, it was found that the noradrenaline has an inhibitory effect on the insulin secretions in the dog and calves' pancreas. It also inhibits the insulin secretion when introduced to isolated islets of the rats, pigs, mice and human pancreas (Kvist et al., 2002); these results are also in agreement with our results.

It is assumed that the noradrenaline inhibits the glucose levels by stimulation of insulin secretion through its direct effect on the alpha-adrenergic receptors located on the islet cells. It was found that the alpha adrenoceptor blockade has counteracted by inhibition of glucose-stimulated insulin secretion by electrical nerve activation. Also, the use of some chemicals alpha adrenergic, e.g. clonidine, has also resulted in the inhibition of insulin secretion, which further supports the role of the noradrenaline in mediating the inhibitory effect of the sympathetic innervation on the islet cells (Al-Muhtaseb et al., 2013).

However, it was also found that the noradrenaline can also stimulate the release of insulin by two different mechanisms: first, through its activation of the islet beta-2 adrenoceptors, which stimulates insulin secretion; and secondly, through a direct action on the alpha cells, probably mediated by both alpha-2 adrenoceptors and beta-2 adrenoceptors, which stimulate glucagon secretion, which might in turn stimulate insulin secretion (Ahren et al., 1990). In conclusion, the net effect of noradrenaline on the insulin secretion depends on the abundance of beta-2 receptors compared to alpha-2 receptors in the islet cells, which might differ under different conditions; it also depends on the action through glucagon.

The results of our study showed that the measurements of the blood glucose levels and the glucose tolerance of the experimental group within different intervals of time after the sympathectomy came in agreement with the stimulatory effect of the sympathetic innervation on the beta cells. A substantial increase in the blood glucose levels was detected after the removal of the sympathetic fibers suggesting the loss of stimulation carried by the adrenergic nerve endings on the islet cells and their insulin secretion (Franco-Colin et al., 2006).

It must be noted, though, that in our surgical procedure of sympathectomy; the removal of sympathetic fibers around the celiac trunk and its main branches is usually accompanied with the cutting of vagal nerve fibers too. This loss of parasympathetic innervations may affect the results. Therefore, the morphologic changes in the exocrine and endocrine parts of the pancreas may be the result of the effect of sympathectomy and vagotomy, but this is slightly unlikely, since cutting some of the parasympathetic nerve fibers should not have the very same effect of cutting the vagal trunk. These results are in agreement with other studies (Mitrani et al., 2007).

In conclusion, the innervation of the pancreas is made up of postganglionic sympathetic and pre-parasympathetic nerve fibers after synapsing with the intrapancreatic ganglions. So, the pancreas is severely affected by cutting any one of them, as was demonstrated in our experiments. The enteric plexus in its turn controls both the exocrine and endocrine parts of the pancreas, this came in agreement with a study done by Gershon et al. (1994), suggesting that the intrinsic neurons can influence the function of the exocrine and endocrine parts of the pancreas.

The pattern of innervation of the rat pancreas was found very similar to other organs such as the heart. According to Mitchell (1956), the parasympathetic innervation of the heart comes through the vagus nerve, which forms part of the cardiac plexus around the trachea. While the sympathetic fibers that come from cervical sympathetic ganglia joins the cardiac plexus around the trachea as postganglionic fibers. After that the cardiac plexus innervates the heart directly or indirectly through the intracardiac ganglia (Mitchell, 1956).

One of the new approaches for the treatment of juvenile diabetes mellitus is the use of islet transplantation methods. Although the results have been far from satisfactory, two important causes are assured to be responsible for the failed transplantation. First, the failure to re-vascularize the donor islets. Second, the loss of the autonomic innervation of the transplanted islets: an altered innervation pattern was found in grafted islets, which was changed with transplant site (Hirshberg et al., 2003; Mikhalski et al., 2014). These theories are strongly supported by our findings, as the loss of the autonomic innervation of the pancreas has led to a high rate of atrophy and subcellular changes in the cells of the islets of Langerhans, thus, it has dramatically reduced the ability of the beta cells to secrete sufficient amounts of insulin that preserve the glucose concentration in the blood.

Furthermore, this study provides additional evidence that the loss of pancreatic autonomic innervation is also responsible for the ischemia and necrosis observed in the pancreatic tissue in patients who had suffered different kinds of neurogenic shock or stress (Barzilai et al., 1987), who usually develop long-lasting effects of denervation that causes the patient's diabetic symptoms.

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