Microscopic Study of Rat Pancreas after Sympathectomy

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Abstract

Objectives: The aims of the study are to monitor the structural changes in the rat pancreatic tissue aftersurgical sympathectomy and assess its effect on the pancreatic endocrine function.

Materials and Methods: Twelve animals were used in this study. All animals had surgical sympathectomy. The morphological changes of the islets of Langerhans and the pancreatic acini were studied under the light microscope, 2 and 3 weeks after surgery. Glucose tolerance test and fasting blood sugar levels were monitored at different intervals of time after sympathectomy.

Results: The microscopic examination of the pancreatic tissue after sympathectomy showed histological changes, in the form of general atrophy and pancreatic cell degeneration. Furthermore, the fasting blood glucose levels and the glucose tolerance tests were significantly increased.

Conclusions: The results of this study demonstrated that the pancreatic function in rats is dependent on the sympathetic innervations,and the metabolic and histologicalabnormalities were observed after surgical sympathectomy.

The structural changes in the pancreatic acini and the islets of Langerhans after vagotomy and sympathectomy may explain the failure of pancreatic cell transplantation and the metabolic abnormalities that accompany the neurogenic shockin humans.

Keywords: Pancreas, Sympathectomy, Microscopic Study.

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Introduction

Much work has been done on various aspects of the pancreatic tissue innervation on different kinds of species, such as different kinds of rats, dogs, and even the one humped camel.[1,2,3]

In the rat, the pancreas is very small and embedded within the mesentery of the small intestine; the pancreatic duct opens into the duodenum, the same as in humans. The

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pancreatic islets are richly innervated by parasympathetic, sympathetic and sensory nerves.[4,20,21]

One of the first findings of a potential role of the sympathetic nerves in the pancreas was presented in 1940.It was found that sympathetic nerve activation causes microscopical changes in the islets structure.[5]

Later, by the use of fluorescent microscopy techniques, the rich network of adrenergic nerve endings were visualized near the pancreatic islet cells in different species.[6]Moreover, electron microscopic studies showed adrenergic nerve terminals in close association with islet endocrine cells.[5.6]

The adrenergic nerves innervating the islets are postganglionic fibers, whose cell bodies reside in the celiac ganglion or in the paravertebral sympathetic ganglia .The preganglionic nerve fibers originate from nerve cell bodies in the hypothalamus and leave the spinal cord at the level of C8 to L3. The fibers then pass within the lesser and greater splanchnic nerves to reach the paravertebral or celiac ganglia.[7]

The postganglionic fibers pass from the ganglia accompanied by the parasympathetic fibers as mixed autonomic nerves that enter the pancreas along its vessels (branches of the celiac trunk). Moreover, preganglionic sympathetic nerve fibers can also directly enter the pancreas and synapse within the organ itself.[7]

Recently, several important studies have been concentrating on pancreatic transplantation,[8] as well as pancreatic denervation and its complications.[9] The effect of chemical sympathectomy on the rat pancreas has been studied and observed in several studies before this study,[10,20,21] but to our knowledge there is no previous description of the effect of surgical sympathectomy on the rat pancreas.

Therefore, the aim of this study is to investigate the effect of sympathectomy on the metabolic function of the pancreas, by observing the histological changes of the endocrine islets and the exocrine acini after sympathectomy.

Measuring the changes in glucose concentration after and before the procedure, we can establish a firm correlation between the autonomic denervation of the pancreas and the complication encountered in different clinical conditions related to the pancreas such as the failure of the pancreatic transplantations and the diabetic symptoms of patients who suffered an episode of neurogenic shock.

Materials and Methods

Twelve adult rats of both sexes were used in this study, and their body weights ranged from 250-450 gm.

All experiments on the animals were conducted according to the NIH guidelines for animal experiments.

The animals were anesthetized by intraperitoneal injections with Sodium Pentobarbital (25mg/Kg) and divided into three groups.

The first group of 4 animals was the control group; the second groups of four were allowed to survive for two weeks after sympathectomy, while the last group was allowed to survive for three weeks after sympathectomy.

Sympathectomy was performed by cutting the splanchnic nerve fibers projecting to the pancreas, all nerve fibers around the celiac trunk, the right and left gastric arteries, and the

nerve fibers around the renal arteries.

All animals were sacrificed by perfusion methods through the left ventricle of the heart: first by normal saline, and then with 10% formaldehyde fixative.

The pancreas was dissected out, cut into small pieces, and placed in fresh fixative for 48 hours. Tissues were dehydrated in alcohol, cleared in xylene, and then embedded in paraffin wax.

The tissue blocks were cut into 10 µm sections, rehydrated and stained with hematoxylin and eosin, and then examined under the light microscope.

Measurements of fasting blood glucose level at different intervals of time after sympathectomy were done and compared with the control group. Also, glucose tolerance tests were conducted two hours after intravenous injection of 0.5g/kg body weight of glucose in the animals that had survived two or three weeks after sympathectomy.

Results

Microscopic examination of the pancreas of the control group animals showed that the exocrine portion comprises the bulk of the organ, and it is subdivided into lobules by connective tissue septa.

Each lobule consists of closely packed secretory acini. The acinus is composed of several pyramid shaped cells possessing round nuclei (figure1).

The endocrine portion of the pancreas is composed of small spherical clumps of different types of cells - islets of Langerhans - which are richly endowed by capillaries. The islets of Langerhans are haphazardly scattered among the serous acini of the pancreas (figure1).

Measurement of the glucose blood levels and the glucose tolerance test of the control group were normal and it ranged between 3.5-4.5 mmol/liter (table1).

Two weeks after sympathectomy, microscopic examination of the islets of Langerhans showed degenerative changes of the cells. Changes in their nuclear structure were observed such as dentate nuclei. Moreover, degenerations were observed in the islets cells three weeks after sympathectomy as some of the cells had disappeared completely and were replaced by connective tissue and bloody structures (figure2).

The values of the blood glucose level measurements at different intervals of time after sympathectomy show a significant increase in the glucose level in comparison to the control group (table1).

In addition to that, the glucose tolerance test two hours after the injection of glucose in the animals who survived two and three weeks after sympathectomy were also significantly increased (table2).

On the other hand, microscopic examination of the exocrine portion of the pancreas two weeks after sympathectomy showed the development of large vacuoles between the secretory acini cells due to shrinkage of the degenerative of the cells, and the acini were not bounded by a clear cell membrane.

Three weeks after sympathectomy more structural changes were observed, as much more vacuoles were noticed, and some cells appeared without nuclei and indistinctive cellular membrane (figure3).

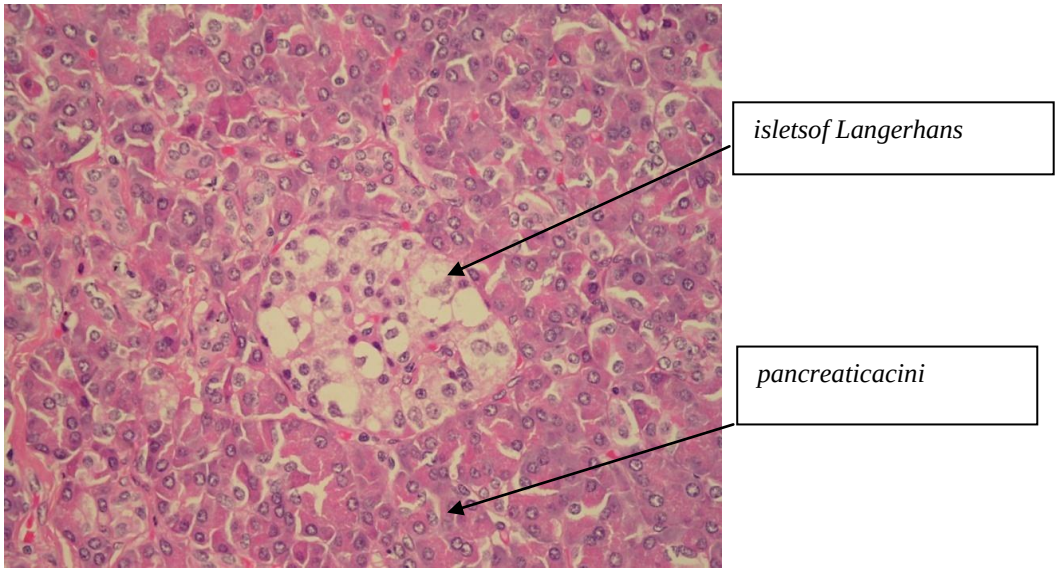

Figure (1): Light micrograph of a section in control rat pancreas showing the endocrine and exocrine cells (H&Ex200).

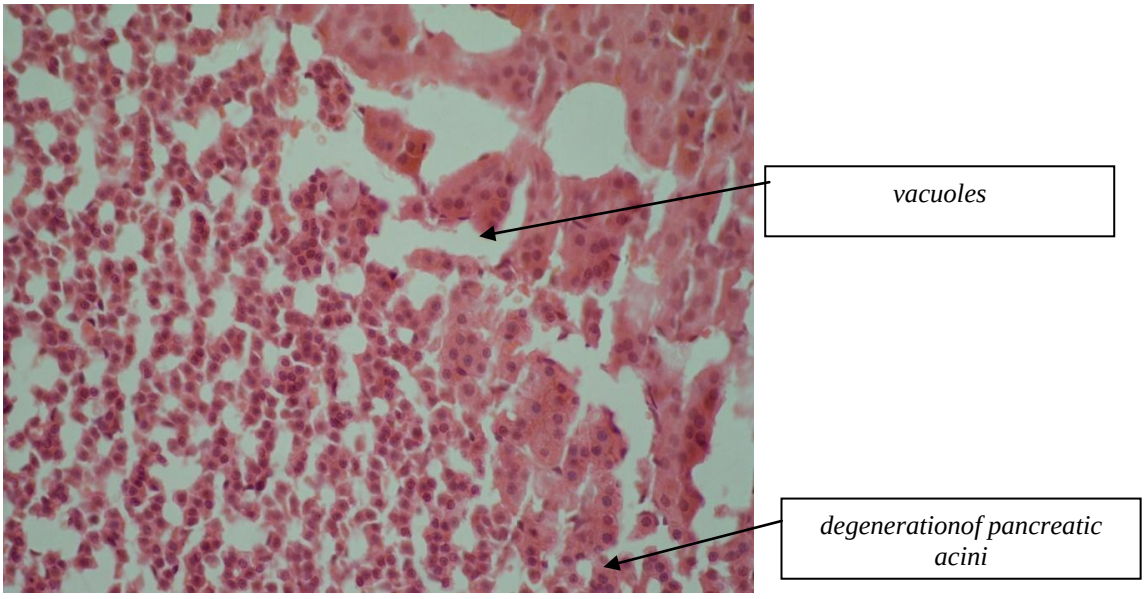

Figure (2): Light micrograph of a section in rat pancreas 2 weeks after sympathectomy showing the degenerative changes in the islets of Langerhans cells; it also shows the formation of the large vacuoles between the pancreatic serous acini due to degenration (H&Ex200).

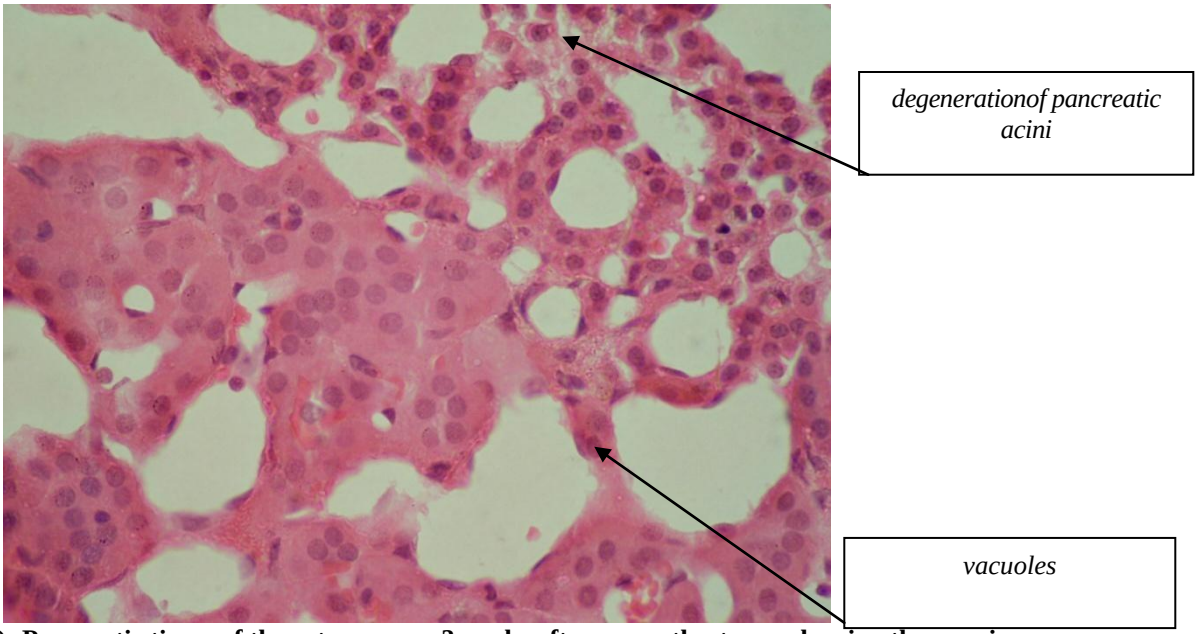

Figure (3): Pancreatic tissue of the rat pancreas 3 weeks after sympathectomy, showing the massive enlargment in the vacuoles and the further degenrative changes in the islets cells (H&Ex400).

Table (1): Glucose blood levels in mmol/L

Table (2): Glucose tolerance test values –two hours after injection of glucose

Period of time after sympathectomy	Control group	Experimental animals
1 week	4.0-4.5 mmol/L	6.3-7.9 mmol/L
2 weeks	4.0-4.5 mmol/L	8.4-10.6 mmol/L
3 weeks	4.0-4.5 mmol/L	8.9-12.6 mmol/L

Discussion

In this study the histology of the control rat pancreas is very similar to the histology of pancreas of other experimental studies.[2,8,11,12]

The correlation between the autonomic innervations and the metabolic abnormalities of the pancreas has been studied and reported in previous studies.[12.20.21]In a study conducted by Lutien in the University of Groningen,[12] different histochemical and immunocytochemical methods were used to observe the intramural sympathetic structure in the non-diabetic and diabetic rats.A 68% reduction in cholinergic cells and a 54% reduction in monoaminergic cells activity weredetected in the diabetic pancreas. These findings support the evidence that the central nervous system plays a crucial role in regulating the glucose concentration in blood.

It must be noted, though, that in our surgical procedure of sympathectomy, the removal of the sympathetic fibers around the celiac trunk

and its main branches is usually accompanied withthe cutting of vagal nerve fiberstoo. This loss of parasympathetic innervations may affect the results.Therefore, the morphological changes in the exocrine and endocrine parts of the pancreas may be the result of the effect of sympathectomy and vagotomy. These results are in agreement with other results.[13]

Several studies have been conducted to investigate the changes in the pancreatic tissue after truncal vagotomy.[14]It is well established that the cholinergic parasympathetic postganglionic nerve fibers have a direct effect on the muscarine receptors of the beta cells of the islets of Langerhans, stimulating them to release insulin.[15]

In a study conducted by Ahren et al.[16] regarding the autonomic regulation of the pancreatic islets secretion, 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 asatisfactory way to detect the direct effects of the sympathetic nerve stimulation on the pancreatic islets without the parasympathetic effects. After stimulation it was found that the noradrenaline has an inhibitory effect on the insulin secretion in the dogs and calves pancreas.It also inhibits the insulin secretion when introduced to an isolated islet of the rat, pig, mice [21] and human pancreas; these results are also in agreement with our results.

It is assumed that the noradrenaline inhibits the glucose-stimulated insulin secretion through its direct effect on the alpha adrenergic receptors located on the islet cells. It was found that the α-adrenoceptor blockade has counteracted the inhibition of glucose- stimulated insulin secretion by electrical nerve activation. Also, the use of some chemical α-adrenergic agonists (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 nerve on the islet cells.

However, it was also found that noradrenaline can also stimulate the release of insulin by two different mechanisms: first, through its activation of the islet β2-adrenoceptors which stimulate insulin secretion and secondly, through a direct action on the alpha cells, probably mediated by both a2-adrenoceptors and b2-adrenoceptors, which stimulate glucagon secretion, thatmight in turn stimulate insulin secretion.[17]In conclusion, the net effect of noradrenaline on the insulin secretion depends on the abundance of the β2-receptors compared to a2 receptors in the islet cells, which might differ under different conditions; it also depends on the action through glucagon.

The results of our study show that the measurements of the glucose blood levels and the glucose tolerance of the experimental group of animals within different intervals of time after the sympathectomy came in agreement with the stimulatory effect of the sympathetic innervations on the beta cells. A substantial increase in the glucose blood levels was detected after the removal of the sympathetic fibres, suggesting the loss of stimulation carried by the adrenergic nerve ending on the islet cells and their Insulin secretion.[20]

One of the new approaches for the treatment of juvenile diabetes is the use of islet transplantation methods, yet the results have

been far from satisfactory. Two important causes are assumed to be responsible for the failed transplantation: first, the failure to revascularize the donor islets and second, the loss of the autonomic innervations of the transplanted islet. An alteration in the innervation pattern was found in grafted islets, which was changed with transplant site.[18]These theories are strongly supported by our findings, as the loss of the sympathetic innervations 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 innervations is also responsible for the ischemia and necrosis observed in the pancreatic tissue in patients who have suffered through different kinds of neurogenic shock.[19] These patients usually develop the long lasting effects of denervation that cause diabetic patient's symptoms.

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الدراسة المجهرية لبنكرياس الجرذ بعد قطع الاعصاب السمبثاوية

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الملخص

الأهداف: دراسة التغيرات التركيبية لأجزاء البنكرياس الصماء وغير الصماء في الجرذ، وذلك بعد قطع الأعصاب السمبثاوية جراحيا، وتقييم تأثير هذه التغيرات التركيبية على افرازات البنكرياس.

الطرق: تمت دراسة التغيرات الشكلية لبنكرياس الجرذ باستخدام المجهر الضوئي بعد اسبوعين وثلاثة اسابيع من قطع الاعصاب السمبثاوية جراحيا. وتم ايضا قياس مستوى الغلوكوز في الدم، وكذلك قياس منحنى الغلوكوز التحملي في الدم على فترات مختلفة بعد عملية قطع الاعصاب السمبثاوية.

النتائج: إن فحص اجزاء البنكرياس الصماء وغير الصماء بالمجهر الضوئي قد أظهرت ضمورا عاما وتآكلا في خلايا البنكرياس بعد عملية قطع الاعصاب السمبثاوية. إن نتائج فحص مستوى الغلوكوز في الدم وكذلك منحنى الغلوكوز التحملي في أوقات مختلفة بعد قطع الاعصاب السمبثاوية كانت مرتفعة ودالة على التغيرات الناتجة.

الخاتمة: إن نتائج هذه الدراسة قد أظهرت ان البنكرياس يعتمد في تغذيته العصبية على الجهاز العصبي المستقل، وإن الاختلالات الأيضية التي لوحظت هي نتيجة مباشرة عن قطع الاعصاب السمبثاوية عن البنكرياس.

النتائج ايضا تفسر أن احد الأسباب المهمة في فشل زراعة خلايا البنكرياس هو فقدان البنكرياس لأعصابه المغذية. وأكثر من ذلك فان التغيرات التركيبية لأجزاء البنكرياس الصماء وغير الصماء الناتجة عن قطع الاعصاب السمبثاوية والجارسمبثاوية قد تفسر كيف أن الضغط النفسي والصدمة العصبية للإنسان من الممكن أن تسبب امراض ومضاعفات أيضية في البنكرياس.

الكلمات الدالة: البنكرياس، قطع الاعصاب السمبثاوية، دراسة مجهرية.