MORPHOMETRIC ANALYSIS OF PANCREATIC ISLETS OF RATS AFTER SUBDIAPHRAGMATIC VAGOTOMY

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UDC 616.833.191.9-089.85-092.9-07:616.37-091-07

Analysis of the morphometric data showed that seven days after vagotomy significant disturbances arise in the pancreatic islets, mainly affecting the B cells. In the late stages (45 and 90 days) after the operation some morphological and functional normalization is observed, but full recovery does not take place.

KEY WORDS: Vagotomy; pancreatic islets; morphometry.

As a result of the study of the effect of subdiaphragmatic vagotomy on the structure and function of endocrine cells of the pancreatic islets [1, 5-7, 13, 14] it has been concluded that the vagus nerve plays an important role in the regulation of the functions of the endocrine pancreas. Previous investigations showed that vagotomy depresses the function not only of the insular apparatus [2], but also of other organs participating in carbohydrate metabolism [3, 10].

The object of the present investigation was to study the principles governing morphological and functional changes in the pancreatic islets at various times after bilateral subdiaphragmatic vagotomy.

METHODS

Experiments were carried out on 150 male albino rats weighing 120-140 g. Under ether anesthesia bilateral subdiaphragmatic vagotomy was performed on 75 animals. The rats were killed 7, 45 and 90 days later in a fasting state, in the morning, and 1, 2, 3, and 6 h after administration of 20% glucose solution by gastric tube in a dose of 2 g/kg body weight. Five control and five vagotomized animals were used at each time. The pancreas was fixed in Bouin's fluid. For quantitative analysis of the secretory granules in the B cells paraffin sections 5 μ thick were stained with aldehyde-fuchsin by Gomori's method and subjected to densitometric analysis by means of the "Microvideomat" microscope (from Opton). The thickness of the sections was measured on the ORIM-I microscope [4]. The number of A and B cells, the dimensions of their nuclei, and the areas of islet tissue were determined in serial sections stained with aldehyde—fuchsin and Halmi's mixture; D cells were revealed by an impregnation method [11]. To compare the relative proportions of islets with different numbers of cells, they were divided into classes [12]. The number of cells in one "average" islet was found by dividing the total number of cells by the number of islets, namely 400 [9]. The relative total areas occupied by cells and also by other structures in the islets were determined with the aid of a morphometric grid. The nuclei were measured by means of a screw-adjusted ocular micrometer. The volumes of the nuclei were calculated by the equation for an ellipsoid of rotation [8]. The results were subjected to statistical analysis.

RESULTS

The endocrine apparatus of the rat pancreas consists of circular pancreatic islets. B cells, the secretory granules of which stain with aldehyde-fuchsin, are central in position, and are arranged around the sinusoidal capillaries. A cells are at the periphery of the islet. The D cells lie between the A and B cells and are characteristically triangular in shape.

Department of Histology and Embryology, Faculty of Internal Medicine, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 11, pp. 609-611, November, 1979. Original article submitted March 2, 1979.

0007-4888/79/8811-1357$07.50

TABLE 1. Quantitative Data for Cell Composition of Pancreatic Islets of Rats

Group of animals		Total number of cells per average islet	No. of A cells		Number of B cells		Number of D cells		Ratio B/A
			absolute	%	absolute	%	absolute	%	
Control		61,98±1,25	12,06±0,36	19,5	45,01±1,15	72,6	4,91±0,37	7,9	3,73
Vagotomy 7 days		53,36±1,08	12,68±0,26	23,8	35,66±0,96	66,8	5,02±0,45	9,4	2,81
	P	<0,001	>0,05	—	<0,001	—	>0,05	—	—
Control		62,65±1,21	12,02±0,37	19,2	45,67±1,15	72,9	4,96±0,28	7,9	3,79
Vagotomy 45 days		55,46±1,13	12,38±0,26	22,3	38,11±0,96	68,7	4,97±0,37	9,0	3,08
	P	<0,01	>0,05	—	<0,01	—	>0,05	—	—
Control		67,71±1,21	12,42±0,32	19,5	46,44±1,17	72,9	4,85±0,32	7,6	3,74
Vagotomy 90 days		58,21±1,25	12,58±0,28	21,6	40,62±1,09	69,8	5,01±0,52	8,6	3,23
	P	<0,05	>0,05	—	<0,05		>0,05	—	—

TABLE 2. Results of Morphometric Study of Pancreatic A and B Cells (M ± m)

Index	Control	Vagotomy 7 days	P	Vagotomy 45 days	P	Vagotomy 90 days	P
Volume, μ^3							
of A cells	301,2±4,4	283,6±7,6	>0,05	320,3±7,3	>0,05	318,2±8,2	>0,05
of B cells	358,9±3,7	418,2±8,7	<0,01	458,6±11,6	<0,01	378,4±9,4	>0,05
Volume of nucleus, μ^3							
of A cells	92,9±1,19	84,8±1,26	<0,05	108,1±1,22	<0,01	96,2±1,43	>0,05
of B cells	106,9±1,18	126,4±1,58	<0,01	154,2±2,31	<0,01	121,0±2,27	<0,01

On the seventh day after vagotomy the islets were no longer compact, the lumen of the sinusoidal capillaries was dilated, and the number of erythrocytes in them considerably increased. There were few secretory granules in the B cells of the fasting animals. The decrease in their number 1 h after administration of glucose was less marked than in the control. During the next 2, 3, and 6 h the accumulation and storage of secretory granules remained below the characteristic level for the control animals. Morphometric analysis showed that the total area of islet tissue was reduced by 21%. The number of cells in one "average" islet was reduced. Meanwhile, as Table 1 shows, there was no significant change in the number of A and D cells. The number of B cells, however, was reduced, with a corresponding decrease in the B/A ratio. The dimensions of the B cells were increased, but at the same time, other cells were seen which were much smaller in size and contained pycnotic nuclei. Comparison of the volumes of the cell nuclei in the fasting animals showed that the volume of the nuclei of the B cells in the experimental animals was increased whereas that of the A cells was reduced (Table 2). The relative percentages of islets of different classes were altered. Very large islets were less frequently found than in the control group, the number of class IV islets showed considerable changes, and most islets belonged to class III. Counting the number of islets in an area of 5 mm^2 showed a decrease in the vagotomized animals (4.6 ± 0.3 compared with 5.7 ± 0.2 in the control; $P < 0.05$).

The sinusoidal capillaries in the islets, as before, were dilated 45 days after subdiaphragmatic vagotomy. The number of secretory granules in the B cells in the fasting state was smaller than in the control. After administration of glucose there were much smaller fluctuations in the number of secretory granules than in the control animals. The total area of islet tissue increased and reached the initial level. The number of cells in an "average" islet was still reduced, but the B/A ratio was higher than 7 days after vagotomy. The mean volumes of the B cells and their nuclei were increased (Tables 1 and 2). Nuclei with twice the normal volume were detected. Classes III and IV of islets had the highest relative percentages, and the number of class V islets was increased to 4% after the operation compared with the number on the 7th day. Among the acinar parenchyma there were islets consisting of two to four cells, the cytoplasm of which stained intensively with aldehyde—fuchsin. The number of islets in an area of 5 mm^2 was 5.2 ± 0.2 (compared with 5.8 ± 0.2 on the control; $P > 0.05$).

After 90 days the pancreatic islets were indistinguishable in structure from the control. The number of secretory granules in the B cells in the fasting state reached the initial figures. However, after glucose loading disturbances of liberation and accumulation of the se-

cretory granules were observed. The B/A ratio was appreciably increased. The total area of islet tissue was indistinguishable from the control. The mean volume of the nuclei of the B cells was increased. As regards the number of islets per unit area, no significant difference was found.

The experimental results showed significant morphological disturbances of the pancreatic endocrine apparatus 7 days after vagotomy, mainly on account of changes in the B cells, with a consequent change in the hormone profile, reflected in the development of hyperglycemia in the animals after vagotomy [3, 5]. The secretory process in the B cells was disturbed. Later (45th and 90th days) complete morphological and functional recovery did not take place. The results show that the vagus nerve plays an important role in maintaining the structural integrity of the pancreatic endocrine apparatus.

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