

JAN 00325

Preganglionic innervation of the pancreas islet cells in the rat

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(Received July 6th, 1983)

(Revised version received October 4th, 1983)

(Accepted November 1st, 1983)

Key words: rat – endocrine pancreas – islet of Langerhans – β -cells – preganglionic
somata – CNS

Abstract

The position and number of preganglionic somata innervating the insulin-secreting β -cells of the endocrine pancreas were investigated in Wistar rats. This question was approached by comparing the innervation of the pancreas of normal rats with the innervation of the pancreas in alloxan-induced diabetic animals. The presumption was made that alloxan treatment destroys the β -cells of the islet of Langerhans and results in a selective degeneration of the β -cells innervation. Cell bodies of preganglionic fibers innervating the pancreas were identified by retrograde transport of horseradish peroxidase following pancreas injections. It was found that 25% of the cells innervating the pancreas in the left dorsal vagal motor nucleus, 50% of the cells in the ambiguus nucleus and 50% of the cells innervating the pancreas, that originate in segments C₃-C₄ of the spinal cord, fail to become labeled after alloxan treatment. The position and distribution of these cell groups are described in detail and are assumed to be involved in preganglionic β -cell innervation. A second cell population in the ventral horn and intermediolateral column of the segments T₃-L₂ of the cord also was labeled in normal rats and was not affected by the alloxan treatment. These thoracic cell groups are thus considered as sympathetic preganglionic somata that maintain direct connections to the pancreas. Additional pre-

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liminary information is presented dealing with the general aspects of sympathetic and parasympathetic organization of the pancreas innervation.

Introduction

There is an increasing amount of evidence that sympathetic and parasympathetic components of the autonomic nervous system originating from the mammalian hypothalamus play an important role in the output system for the endocrine control of glucose homeostasis. It has been demonstrated [3,32,34,35] that electrical or chemical stimulation of the lateral hypothalamic area (LHA) results in an increased secretion of insulin, without a significant change in glucagon levels; parasympathetic mechanisms are involved. Stimulation of the ventromedial hypothalamic nucleus (VMH) elicits a rise in plasma glucagon levels mediated by sympathetic components of the autonomic nervous system [3,8,11,32,34,35]. It was demonstrated by Bereiter et al. [1] that at lower brainstem levels electrical stimulation of the ambiguus nucleus and surrounding neuropil causes a sharp rise in insulin release as a result of parasympathetic activation. It was reported by several authors that stimuli applied to the vagal nerve peripherally have a strong effect on insulin release, whereas stimulations of the sympathetic fibers of the splanchnic nerves give rise to an increased glucagon secretion and a suppression of insulin release [2,4,5,12,18,24]. On the basis of these studies it has been assumed by several authors [27,31] that there must be a circuit between hypothalamus and endocrine pancreas underlying the observed physiological processes. This neural or brain circuit should include lateral and ventromedial hypothalamic nuclei and the vagal and splanchnic nerves of the parasympathetic and sympathetic autonomic divisions, respectively.

The present investigation has been undertaken to elucidate the position in the central nervous system of the preganglionic parasympathetic cell bodies innervating the insulin-releasing β -cell in the endocrine pancreas. This question was approached by an indirect method that enabled us to compare the central locus and numbers of cells innervating the pancreas of normal rats, with the innervation of the pancreas of alloxan-diabetic animals. The preassumption made was that alloxan treatment results in complete destruction of the β -cell in the islets of Langerhans and that this eventually leads to a degeneration of the neural fibers which innervate the B-cells. The origins, types and numbers of cells innervating the pancreas in control and alloxan-treated animals were analyzed by means of retrograde transport of horseradish peroxidase. Preganglionic parasympathetic cell groups involved in β -cell innervation were identified in left motor vagus nucleus, bilateral ambiguus nuclei and segments 3 and 4 of the cervical spinal cord.

Apart from data on localization of parasympathetic somata the present results also suggest an organization of the sympathetic component of pancreas innervation that differs considerably from the hitherto current, classical views. To provide additional evidence concerning the general aspects of pancreas innervation a number of experiments were performed in which we combined cellular degeneration with use of histochemical techniques.

Materials and Methods

In the present investigation 48 animals were used. All experiments were performed on male Wistar rats weighing between 300 and 400 g. Of the total of 48, 15 preliminary experiments were needed to develop a highly reproducible operation procedure with an optimized sensitivity for the retrograde tracer. The data presented here are based on results obtained from the remaining 33 animals. In the first part of the study experiments were carried out on 26 rats to investigate the position of somata of preganglionic neurons innervating the pancreas. In the second part of the study 7 more animals were treated in various ways to throw light on the general aspects of total autonomic innervation of the pancreas.

The 33 animals of the first part were divided into a "normal" group ($n = 10$) and an alloxan-diabetic group ($n = 16$). The rats of the diabetic group were starved for 24 h prior to an intraperitoneal injection of 5% alloxan in sterile distilled water in a dose of 15 mg/100 g body weight. Following this injection the animals were food-deprived for another 24 h. During one week the daily urine production was measured and glucose excretion determined with Lilly's M73 testtape. The rats were considered as diabetic when they produced about 100 ml urine/24 h with a glucose concentration of 0.5%. Until further experimental treatment the diabetic animals received subcutaneous injections of 6 I.U. of long-acting protamine zinc insulin (Organon, Oss, The Netherlands) every other day. The survival period after alloxan treatment was at least 5 weeks in order to effect complete degeneration of the β -cell and its innervation.

The animals of both normal and diabetic groups then received an injection of horseradish peroxidase (HRP) into the pancreas. This was performed under ether anaesthesia according to the method described by Cyprian Weaver [7]. This procedure involved an injection into the pancreas, via a silicone rubber catheter inserted into the common bile duct, of 35 μ l of a 25% HRP (Boehringer, Grade I) solution in saline containing 1.6 mg% hyaluronidase and 1.6 mg% collagenase. Ligation of the bile duct and of the ampulla, where the bile duct ends in the duodenum, prevented leakage of the tracer from the pancreas. The hyaluronidase and collagenase were added to the HRP solution to overcome perineural barriers like connective tissue components and basal laminae in order to obtain optimal tracer uptake by nerve terminals [16,19]. Comparison with preliminary experiments in which these enzymes were not added to the tracer solution showed that the enzymes mentioned greatly improve the density of the labeling and allow the positive detection of approximately 20% more cells. Following a survival period of 28 h the animals were perfused transcardially with a pre-rinse consisting of 0.8% NaCl, 0.8% sucrose and 0.4% D-glucose in 0.05 M phosphate buffer (pH = 7.4) to which 8 I.U. heparin/ml was added. This prerinse was followed by the fixation fluid made up of 0.5% paraformaldehyde, 1.5% glutaraldehyde and 4% sucrose in phosphate buffer. Subsequently brain and spinal cord were removed and stored overnight at 4°C in buffered 30% sucrose. The spinal cord was removed in two parts by means of an anterior incision between vertebrae C₁ and C₂, an intermediate incision between the third and fourth thoracic vertebrae and a posterior cut between the thoracic vertebrae 12 and 13. We

thus obtained an anterior part of the cord containing the segments C₁ to T₃ and a posterior part comprised of segments T₄ to L₂ [14]. Forty- μ m transverse sections of the brainstem and horizontal sections of the spinal cord were cut on a cryostat microtome and collected in chilled 30% sucrose plus 30% ethyleneglycol in buffer. Every second section was stained for HRP according to the benzidine-2HCl method of De Olmos and Heimer [10] yielding superior sensitivity. In some experiments the remaining sections were treated by the TMB procedure of Mesulam [23]. All sections were carefully scanned and position and numbers of labeled somata recorded. From the group of 'normal' animals 3 cases were discarded because of technical reasons such as poor fixation. From the 'diabetic' group 9 cases either did not survive the alloxan treatment or the subsequent HRP injection.

As will be pointed out in the following section of this paper the wide spread labeling in lower medulla regions posed questions regarding the structural organization of sympathetic vs parasympathetic organization. So, in the second part of this study some preliminary experiments were carried out that provided additional information on the autonomic innervation of the pancreas.

In two rats HRP was injected into the pancreas following a unilateral transection of the vagal nerve. Individuals of another group of 5 animals were tested for acetylcholinesterase (AChE) activity within the dorsal motor nucleus of the vagal nerve (DMV) in order to assay degenerative changes in cellular activity following alloxan treatment. In the latter test situation 3 alloxan-diabetic animals were studied. Those results were compared with AChE activity in the DMV in a normal animal and with the activity of AChE in a unilaterally vagotomized rat. In all these animals activity of AChE was demonstrated using the modified Koelle technique according to Navaratnam et al. [25].

As described above all cell counts were made in every second section of brain and cord. So the total numbers thus recorded were multiplied by two and the formula of Koningsmark [20] applied for cell size and section thickness to correct for double counting. The numbers thus obtained are the corrected total cell numbers.

Results

Retrograde labeling of somata following HRP injections into the pancreas of normal rats

After injection of horseradish peroxidase into the normal untreated pancreas retrogradely labeled somata occurred in 5 loci of the brainstem and the spinal cord. In the lower brainstem labeled cells were detected in the dorsal motor nucleus of the vagal nerve (DMV) (Fig. 1), in the nucleus ambiguus (AMB) (Fig. 2) and in the nucleus reticularis lateralis (RL). Of these medullary nuclei the DMV appeared to be the most important source of pancreas innervating cells in a quantitative sense. There appeared, however, to be a considerable difference between labeling in left and right DMV. In the left DMV an average of 1697 labeled somata were recorded, in the right DMV the total average number was 962 cells.

Much less numerous were the labeled cells in the ambiguous nuclei. Besides, there

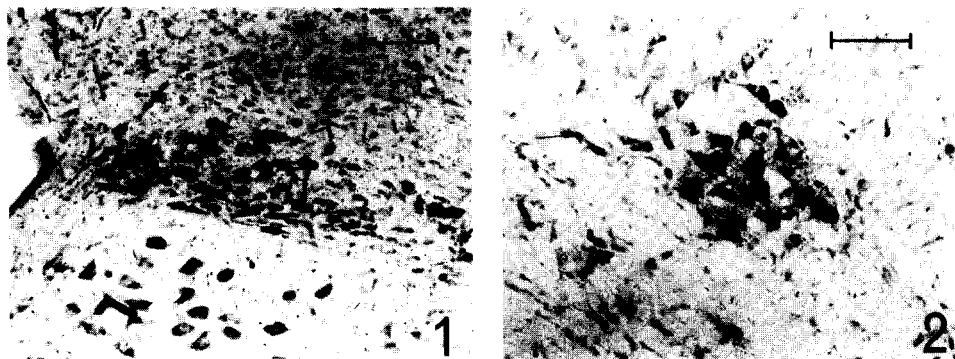


Fig. 1. Photomicrograph of retrogradely labeled somata in the dorsal motor vagal nucleus following an HRP injection into the pancreas. Note that labeling is more numerous in the medial aspects of the nucleus.

Fig. 2. Photomicrograph of HRP-labeled cells in the ambiguous nucleus after tracer injection into the pancreas. Scale bar in Figs. 1 and 2 is 100 μ m.

was no significant difference between numbers in nuclei of the left and right side of the brain. In the left AMB 133 cells were counted and in the right AMB 142 labeled somata were seen. Although inconspicuous, it is worth mentioning that small numbers of HRP positive perikarya were detected in the lateral reticular nuclei. The number of cells labeled in the RL was never more than 20 on each side of the medulla. However, the impression was gained that the RL cells labeled might be regarded as displaced ambiguous cells.

In the spinal cord two bilateral columns of labeled somata appeared following tracer injections into the pancreas. An anterior column of labeled cells was localized in the cervical segment C₃ and C₄ and consisted of 214 cells on the left side and 232 cells on the right side of the cord (Fig. 6). A posterior column of HRP-positive cells was observed in the ventral horns of the cord segments from thoracic 3 to lumbar 2 (Fig. 7). Here too, there was an almost equal bilateral distribution of cells, i.e., 863 left and 880 right.

Retrograde labeling of somata following HRP injections into the pancreas of alloxan-diabetic rats

Injections of horseradish peroxidase in the diabetic animals resulted in labeling of somata in the same CNS sites as after injection in normal animals, but with considerable quantitative differences. Cell counts showed that the number of labeled somata in the left DMV totalled an average of 1247. This means a decrease in average numbers of 450 cells or 26.5%. The DMV of the right side of the brainstem showed considerably less labeling. Here we counted 868 labeled somata. So for the right DMV the decrease of labeled cells was only 94 neurons or 9.8%. Labeling in the ambiguous nucleus in the diabetic cases is bilaterally almost equal: in the left AMB 71 cells and in the right AMB 73 labeled perikarya. Compared to the normal cases the AMB labeling showed a relatively strong decrease in labeling: 46.6% and

TABLE I

TOTAL AMOUNTS OF LABELED SOMATA IN VARIOUS CNS STRUCTURES IN AVERAGE NUMBERS

Given are the mean numbers of cells counted in every second section. In parentheses the numbers of cells calculated with corrections for double-counting. Differences were statistically tested with the Mann-Whitney U-test. $P < 0.05$ was considered significant (S), larger values non-significant (N.S.).

| Structure | Normal animals (n = 7) | Diabetic animals (n = 7) | Difference | % | Test |
|------------------------------------|---------------------------|-----------------------------|-------------|--------|------|
| DMV (L) | 1 286 (1 697) | 945 (1 247) | - 341 (450) | - 26.5 | S. |
| DMV (R) | 729 (962) | 658 (868) | - 71 (94) | - 9.8 | N.S. |
| AMB (L) | 101 (133) | 54 (71) | - 47 (62) | - 46.6 | S. |
| AMB (R) | 108 (142) | 55 (73) | - 54 (69) | - 48.6 | S. |
| C ₃ -C ₄ (L) | 243 (214) | 100 (88) | - 143 (126) | - 58.8 | S. |
| C ₃ -C ₄ (R) | 263 (232) | 121 (107) | - 142 (125) | - 53.8 | S. |
| T ₃ -L ₂ (L) | 757 (863) | 856 (976) | + 99 (113) | + 13.0 | N.S. |
| T ₃ -L ₂ (R) | 772 (880) | 805 (918) | + 33 (38) | + 4.9 | N.S. |

48.6% for left and right side, respectively. In the diabetic animals labeled cells also were found in the lateral reticular nucleus; however, in the same small numbers that do not permit a meaningful comparison.

In the spinal cord of the diabetic animals labeling also occurred in an anterior and a posterior column. Labeling in the anterior column differed considerably from labeling in the normal cases. The diabetic animals showed a decrease in cell count. The change on the left side of the cord in C₃-C₄ was from 214 in the normal to 88 in the diabetic group which is a decrease of 58.8%. On the right side the difference was 232 (normal) and 107 (diabetic) which is a decrease of 53.8%. Conspicuous was the labeling in the posterior column within the cord (T₃-L₂). On the left side 976 and on the right side 918 cells were found labeled in this area. Compared to the numbers of labeled somata in the normal group this is a (non-significant) increase of 13% on the left and 4.9% on the right. The alloxan treatment apparently had no effect on the cellular labeling in the latter locus. The moderate increase in cellular labeling is probably a matter of intraspecific variability. So, in summary it may be concluded that alloxan treatment did result in destruction (and consequently a lack in retrograde labeling following pancreatic tracer injection) of about a quarter of the pancreas-innervating cell group in the left DMV, whereas about half of the cell populations in the ambiguous nucleus and C₃-C₄ segments of the spinal cord became affected. The cell groups in T₃-L₂ segments of the cord involved in pancreas innervation did not seem to be affected by alloxan treatment.

Topographic and structural characteristics of pancreas innervating cell groups

Labeling in the dorsal motor vagus nucleus and ambiguous nucleus. The largest number of cells innervating the normal and diabetic pancreas were found in the DMV. Usually the shape of these cell bodies was bi- or multipolar with an average diameter of 20 μ m. Cellular labeling occurred at all levels bilaterally of this nucleus,

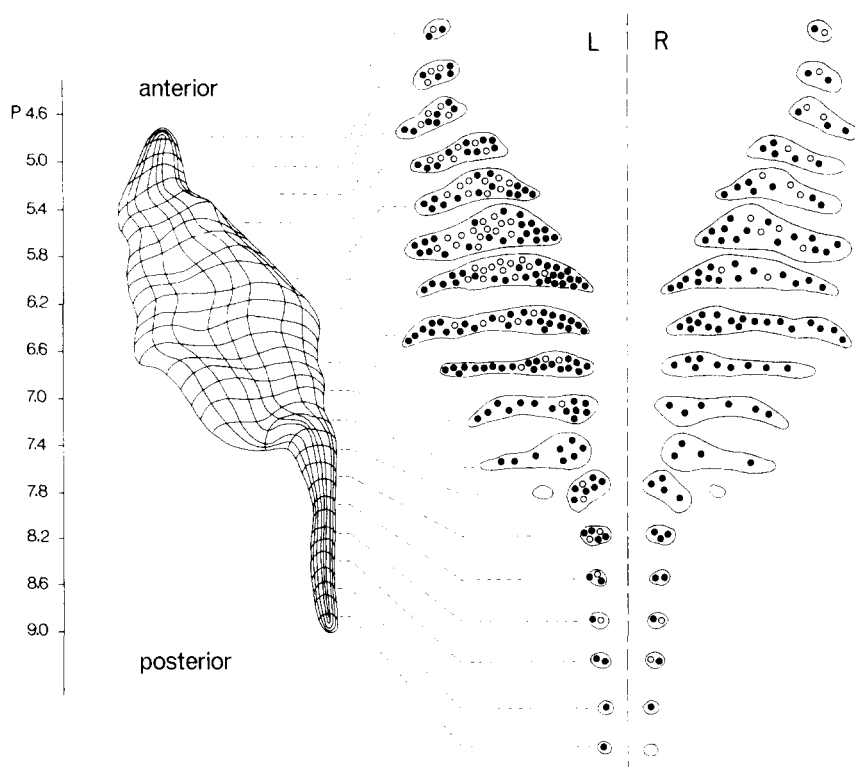


Fig. 3. Dorsal view of a stereometric reconstruction of the left dorsal motor nucleus of the vagal nerve (DMV) as re-defined by Dennison et al. [9]. The scale left indicates the anterior-posterior coordinates as posterior to interaural line as given by Pellegrino et al. [26]. The two columns at the right give a series of transverse sections of left and right DMV in which is indicated retrograde labeling of somata following pancreas injections of horseradish peroxidase. Open and filled circles together represent the position and distribution of labeled cells as they appear after pancreas injections in normal animals. The filled circles alone represent retrograde labeling of somata after injections in diabetic animals. Thus, the open circles illustrate position and distribution of preganglionic somata that fail to become labeled in diabetic animals and so apparently represent cell groups innervating the pancreatic β -cells.

which has the shape of a dorsoventrally flattened body provided with a short rostral and a large caudal tail (Fig. 3) which is identical to the re-defined DMV of Dennison et al. [9]. The nucleus measured a total length of about 4.4 mm. The largest width and also largest number of labeled cells were found at the level of the area postrema. To illustrate the position of labeled somata within the DMV and the distribution of cells over the entire nucleus the DMV was divided into segments of 240 μ m, both in the normal and the diabetic animals. The position of labeled cells has been plotted in a series of transverse sections through the DMV at 240 μ m intervals (Fig. 3). In each section of the DMV each symbol, i.e. a filled or open circle, represents approximately 8 labeled somata. Filled and open circles together indicate the position of labeled somata as they appear after HRP injection in normal animals. The open circles illustrate the position of cells that did become labeled in the normal cases, but

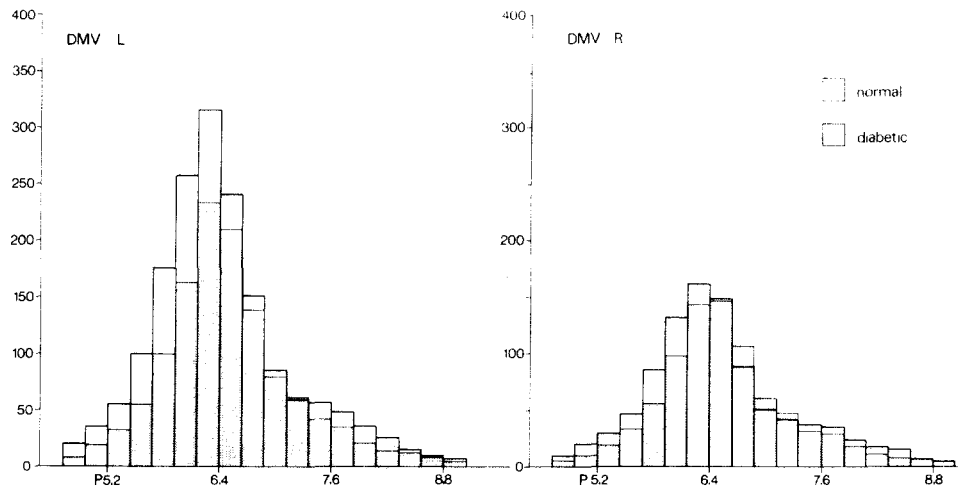


Fig. 4. Histograms with numbers of labeled cells in left and right DMV after pancreas injection of HRP in normal and diabetic rats. The histogram consists of a series of columns representing data of 240 μ m sections of the DMV at anterior-posterior levels indicated on the ordinate. Numbers of labeled somata are given on the abscissa. The open column give numbers of cells in normal animals, the stippled part of each column the numbers in diabetic rats. The graph clearly indicates that the absolute decrease of labeled cell numbers occurs in the left DMV at an anterior-posterior level approximately between P5.6 and P6.4 which corresponds to the level of the area postrema.

failed to become labeled after tracer injections in the alloxan-diabetic cases. The open circles then give the position of the presumably degenerated preganglionic cells innervating the destroyed β -cells of the endocrine pancreas.

The quantitative differences of cellular labeling after HRP injections in the alloxan-treated diabetic animals have been graphically shown in Figs. 4 and 5. In these figures numbers of labeled cells as determined for 240 μ m segments of the DMV (Fig. 4) and ambiguous nucleus (Fig. 5) are given as open columns for the normal animals and dotted columns for the diabetic cases.

As can be concluded from Fig. 3 normal pancreas-innervating preganglionic cells appear over the entire length of the DMV, although the density in the more medial aspects of this nucleus is slightly higher than in the lateral parts. Moreover, the labeling in the left DMV by far outnumber the right DMV labeling. The open circles in Fig. 3, indicating the position of cells that do not become labeled in the diabetic cases, are predominantly found in the central parts of the left DMV at the more anterior levels. In the right DMV the number of HRP-positive cells that do not appear after alloxan treatment is relatively small and is not statistically significant, whereas the numbers of cells in the left DMV is significantly smaller in diabetic animals than in normal cases. The quantitative changes in HRP uptake after alloxan treatment are also apparent from the histograms in Fig. 4. Numbers of cells determined for 240 μ m segments of the DMV are given for normal and diabetic animals. In the left DMV almost all segments in the diabetic cases show a decrease in numbers of cells, but the decrease in absolute numbers is the largest at levels from

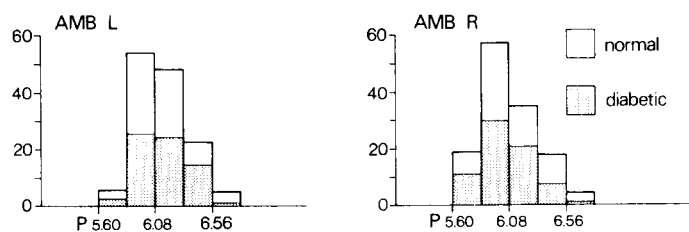


Fig. 5. Histogram with numbers of labeled cells in left and right ambiguous nucleus after pancreas injections in normal and diabetic rats. See for further explanation Fig. 3. Decrease of labeled cells in diabetic animals is almost equally distributed at all levels of both left and right ambiguous nuclei.

anterior-posterior [26] P5.2-P6.4, which is the level that coincides with the area postrema.

The histograms for cellular labeling in the ambiguous nucleus (Fig. 5) show an almost equal reduction on the left and right side. The AMB situated between coordinates P5.60 and P6.80 reveal a significant decrease of cellular labeling in all anterior-posterior segments on both left and right sides in alloxan-diabetic animals.

Labeling in the spinal cord. In the spinal cord two columns of cellular labeling occurred after injections of HRP in the pancreas. One column was localized in segments C₃ and C₄ and a second population in segments T₃ to L₂.

The anterior cell group in C₃-C₄ consists of large cells occurring bilaterally in the ventral horn of the cord (Fig. 6). These cells that are very reminiscent of the α -motoneuron type, are usually multipolar, carrying dendrites and measure up to 50 μ m in diameter. The cells, however, are not spread all over the ventral horn but form a very dense column of tightly packed somata. Cellular labeling in the diabetic animals clearly indicate that cellular labeling in this cell group was strongly affected by the alloxan treatment. In the diabetic group the numbers of labeled somata were half the number in the normal cases, thus showing a significant decrease of more than 50%. Moreover, the labeling observed in the diabetic cases usually was much lighter than in the normal animals.

The second column of spinal labeling after pancreas injections occurred in segments T₃-L₂ (Fig. 7). Cellular labeling in this segments was localized in two areas. Large and midsize efferent cells were found labeled in the ventral horns. These cells varied somewhat in size and measured from 20 to 45 μ m although the majority of the ventral cells were of the larger type. A second group of labeled somata occurred at midlateral levels (Fig. 7). These cells usually were smaller than the ventral somata, measuring about 15 μ m in diameter and often were more spindle-shaped. Moreover, the cells at this level were found to be more spread in the horizontal plane. The majority of cells were found in the lateral aspects of the grey but also cells were observed in a more medial position close to the central canal. Although these cells are found at midlateral levels their position does not completely fit the classical position of the intermediolateral column (IML), but at least in part occur slightly more dorsal to the IML region. Conspicuous was the fact that the cellular labeling in this part of the cord did not show a decrease in the diabetic cases, but even in minor

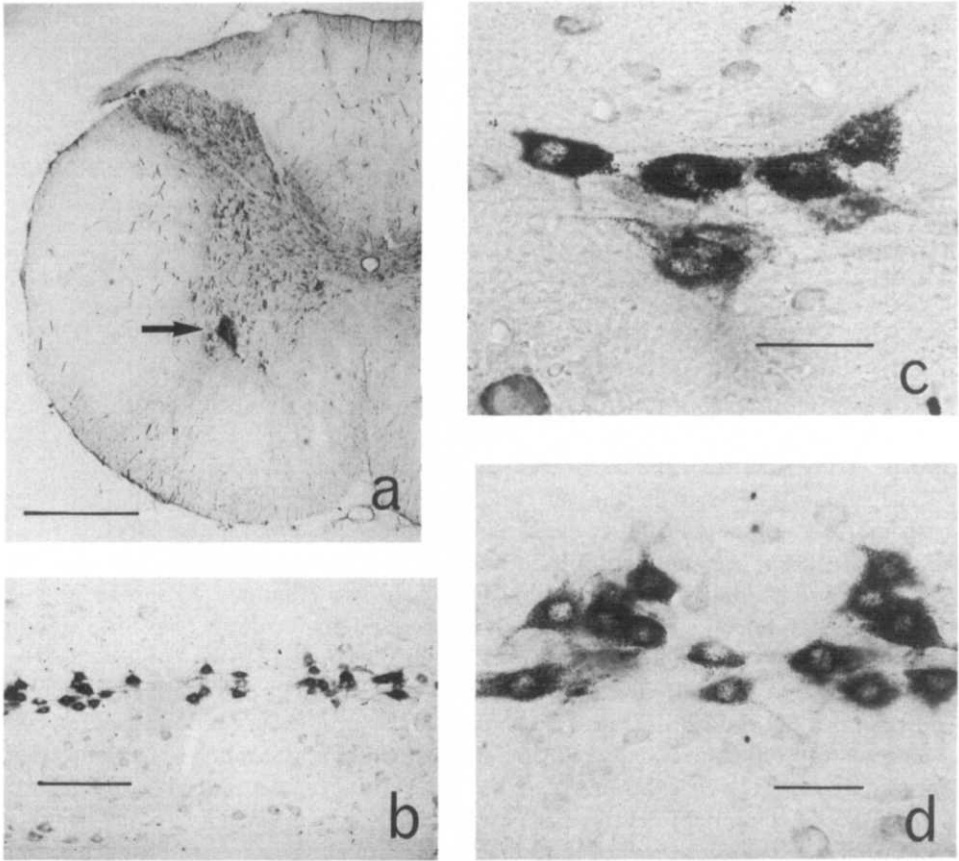


Fig. 6. Photomicrographs of retrograde labeling with HRP in segments 3 and 4 of the cervical spinal cord. a: transverse section with the position of labeled cells in the ventral horn (arrow). Scale bar = 750 μm . b: longitudinal section of the cervical cord showing the well organized column of the labeled somata in the ventral horn. Scale bar = 250 μm . c and d: show the labeled cells in C3-C4 in detail. Section is stained by the TMB procedure [23]. Sections in b-d stained with BDHC [10]. Scale bar in c and d = 50 μm .

increase of average number. This increase, however, was not statistically significant.

Vagotomy experiments. The results described above led us to the conclusion that the labeled areas in lower medulla and C₃-C₄ segments of the spinal cord appear as candidates for preganglionic parasympathetic sites innervating the endocrine pancreatic β -cell. Since the vagal nerve is generally considered as the parasympathetic pathway we have tried to establish whether all of these CNS sites including C₃-C₄ reach the pancreas via the vagal nerve. To answer this question in two animals we studied retrograde CNS labeling following HRP injections in the pancreas subsequent to a transection of the left vagal nerve in the cervical region. As a result of this unilateral vagotomy, retrograde labeling of somata in the left DMV and AMB were absent, but the amount and position of cellular labeling in C₃-C₄ and T₃-L₂ were present as in normal cases. This indicates that the presumed parasympathetic

cell groups in C₃-C₄ are not reached via vagal nerve fibers (at least at cervical levels) but that the axons originating from C₃-C₄ follow a separate peripheral course on their way to the pancreas.

AChE experiments. One of the striking results was the fact that in normal animals an average of almost 1700 cells became labeled in the DMV following pancreas injections. Since we counted that the DMV consisted of a total of

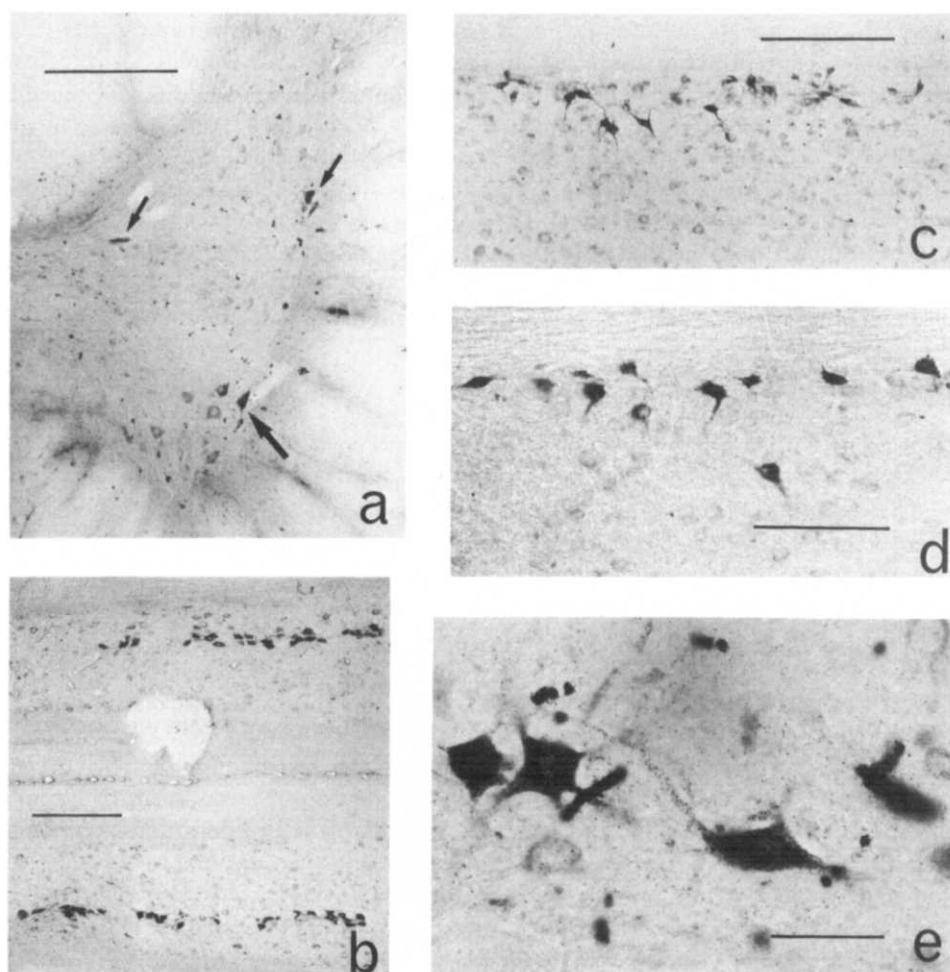


Fig. 7. Photomicrographs of labeled cells after HRP injections in the pancreas in segments T₃-L₂. a: transverse section through the thoracic cord with labeled somata in the ventral horn (large arrow) and in the intermediolateral column (small arrows). Note that some IML cells take a more medial position. TMB stain. b: longitudinal section through the ventral horn with column of large labeled somata. BDHC stain. Scale bars in a and b = 500 μ m. c and d: show labeled somata in the intermediolateral column in more detail in a longitudinal section. Scale bar in c = 200 μ m; scale bar in d = 100 μ m. BDHC stain. e: illustrates in detail the large labeled somata in the thoracic ventral horn. Longitudinal section. BDHC stain. Scale bar = 50 μ m.

approximately 4650 cells this would imply that about 36% of the cells in the left DMV are involved in the innervation of the pancreas alone. In our opinion this high percentage indicates that the individual cells innervating the pancreas might innervate additional visceral target organs. This would imply a considerable axonal collateralization of vagal fibers which means a multiple innervation task for the individual vagal efferent cell. To approach this question we studied the AChE contents of DMV somata in normal, vagotomized and alloxan-diabetic animals. We then reasoned that in case there exists a unimodal innervation task for β -cell innervating preganglionic somata this would be reflected in significant decrease of the number of AChE-containing cells in the DMV in diabetic animals.

In all animals treated for AChE we only counted the somata that displayed a strong activity. In one normal animal 1886 AChE-positive cells were counted in the left DMV. To determine the effect of peripheral axonal damage to the AChE activity of DMV cells in one vagotomized animal that survived for 5 weeks, the number of AChE-containing cells in the left DMV was found to be 320. To study the effect of alloxan-induced degeneration of the β -cell innervation we tested 3 diabetic rats 6 weeks after alloxan treatment. The amount of AChE-positive somata in the left DMV in these animals was shown to be 1805 and did not show any significant decrease.

Discussion

The present data on normal pancreas preganglionic innervation indicate 5 CNS sites, 3 in the lower medulla: dorsal motor vagus nucleus, nucleus ambiguus and lateral reticular nucleus and two in the spinal cord: ventral horn cell populations in segments C_3 and C_4 and a predominantly ventral horn column in segment T_3-L_2 . Most of these brain sites from which pancreatic innervation originates have already been described by several other authors [7,22], although our results show some differences from those reports regarding the detailed position and distribution of labeled somata. Laughton and Powley [22] did not describe the minor labeling in the lateral reticular nucleus of the medulla and also the distribution of labeled cells within the DMV was more restricted in their observations. Moreover, in their study the spinal labeling was found in C_2 and C_3 and that in the posterior spinal column was limited to T_5-L_1 . Furthermore their spinal labeling was confined to ventral horn somata, whereas in our material cellular labeling in T_3-L_2 was also observed in intermediolateral regions. There is, however, a striking discrepancy between our results and those of Cyprian Weaver [7] whose observations are limited to the DMV and AMB. The latter author found labeling in the DMV after pancreas injection predominantly on the right side of the brain. A possible explanation for this striking difference from our observations using Wistar rats may be due to differences in strains of rats studied by others. In alloxan-diabetic rats a significant decrease in numbers of cells innervating the pancreas was observed in the left DMV, bilateral AMB and bilateral C_3-C_4 cell groups of the spinal cord. The latter observations indicate the position of the preganglionic cell groups innervating β -cells that fail to

become labeled by retrograde tracers as a result of selective destruction of the pancreatic β -cell and its efferent parasympathetic innervation.

Although the mechanism of toxic action of alloxan is a matter of dispute [6,28] it is a well documented phenomenon that alloxan has a specific destructive effect on the pancreatic β -cell that after 4 weeks leads to a total or almost total loss of β -cells in the islet of Langerhans [15,17,37] (Fig. 8). The destruction of β -cells and the overall reorganization of the pancreatic islets [17,37] leads to changes in the islet innervation, that have been demonstrated by Shorr and Bloom [33]. They described dystrophy of β -cell contacting nerve terminals in alloxan-treated rats. These peripheral neural changes then eventually would result in either a transganglionic retrograde degeneration of the parasympathetic fiber pathways originating in the preganglionic CNS sites or being confined to a destruction of terminal intramural structures. In both cases the uptake capacity in the pancreas for the applied retrograde tracer would be responsible for the decrease in amount of retrograde labeling observed in the classical parasympathetic sites in the lower medulla oblongata.

An argument for selective destruction of the β -cell and its parasympathetic innervation is also given by the labeling in the thoracic sympathetic segments of the spinal cord in normal and diabetic cases. As stated we did not find a significant change in labeling in these sympathetic regions of the cord after alloxan treatment in contrast to the parasympathetic areas in the CNS.

Although observed before [22] for several reasons the retrograde labeling in the spinal cord following pancreas HRP injections is a rather unexpected finding that

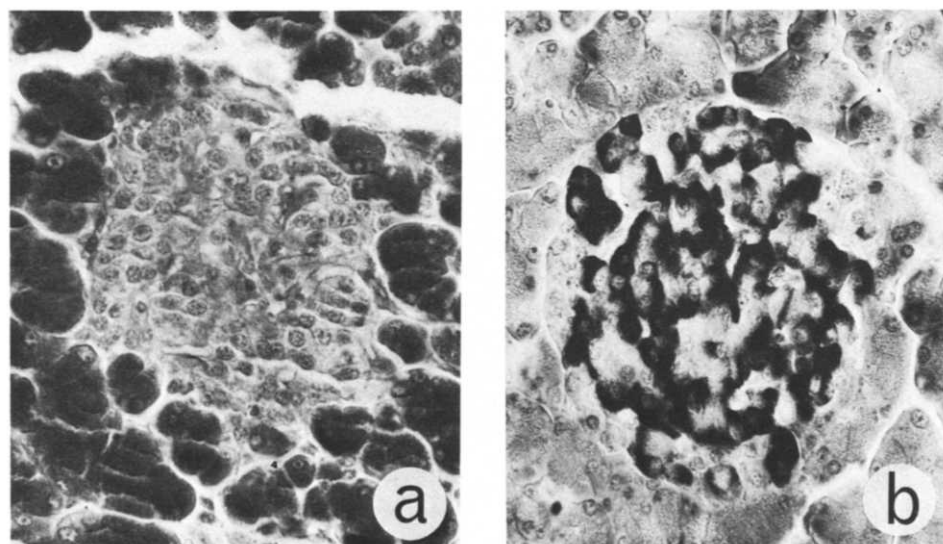


Fig. 8. Photomicrographs of islets of Langerhans in an alloxan-induced diabetic rat (a) and in a normal untreated animal (b). Both sections are stained for the presence of insulin by the aldehyde fuchsin method. Exocrine tissue in a is stained with halmi. Note the absence of stained granula in the diabetic case in a.

does not match the general concept of autonomic organization patterns. Both in the cervical and thoracic segments labeling was observed of large somata of the motoneuron type in the ventral horns that apparently do have a direct connection to their target organ without synapsing in an extramural ganglionic structure. Although this type of innervation is common in the parasympathetic division of the autonomic nervous system this finding is unexpected for the sympathetic branches. It is generally accepted that the adrenal gland receives an uninterrupted sympathetic innervation from the intermediolateral cell group in the thoracic segments of the cord [13]. Such a direct pathway for visceral organs like the pancreas as we found in this study is, however, unknown and cannot be explained by transsynaptic transfer of HRP in the extramural ganglion since HRP is not able to do so. There is, however, evidence that at least in the superior cervical ganglion in rat a number of preganglionic fibers do not end in the ganglion but continue uninterrupted in the postganglionic nerves [29].

Even more puzzling is the labeling of the large neurons in the ventral horns both in the cervical and thoracic segments. It is hard to believe that the observed labeling is the result of an artifact firstly because we are dealing with strong labeling in a well organized pattern. Secondly, the considerable reduction of cellular labeling in the cervical segments in the diabetic animal clearly indicate an autonomic function. The fact that almost none of these large ventral horn cells became labeled after applying HRP to the transected splanchnic nerve [21] indicates that the large cells in the T₃-L₂ in our study became labeled via different pathways. The same conclusion was reached for the cellular labeling in the segments C₃-C₄ which continued to occur after cervical vagotomy.

The AChE experiments in this study in which we did not observe a numerical decrease of AChE containing cells in the DMV after alloxan treatment lead us to the preliminary conclusion that the alloxan-induced degeneration of the β -cell innervation did not reach the preganglionic parent cell bodies in the DMV. One of the possible explanations for this observation might be that the alloxan-induced degeneration may only effect axonal collaterals that cannot cause a total cellular destruction. This would imply that each cell in the DMV by collateralization innervates more than one of the visceral target organs. Although much more evidence is needed numerical data other than ours support this view. It was observed in recent studies [30,36] that a very large proportion of DMV cells project to the stomach wall, which implies that the DMV simply does not contain enough cell bodies to provide a separate innervation for all visceral organs controlled by the DMV. In a current continuation of this study double-labeling experiments are being carried out in this laboratory to study the possibility of multiple innervation of individual DMV cells.

Acknowledgements

We thank Prof. A.H.M. Lohman of the Dept. of Anatomy of Amsterdam Free University for the particular interest he has shown during the course of this study. The work was supported in part by the Foundation for Medical Research FUNGO (Grant 13-46-36).

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