ELSEVIER

0963-6987(95)02019-5

Original Contribution

NORADRENERGIC AND CHOLINERGIC REINNERVATION OF ISLET GRAFTS IN DIABETIC RATS

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☐ **Abstract — Grafted islets become denervated due to the islet transplantation procedure. The aim of the present study was 1) to examine whether islet grafts in the liver, the spleen, and under the kidney capsule in rats become reinnervated following the transplantation and experimental procedures used in our laboratory, 2) whether there is any difference in reinnervation at these different sites, and 3) how these results relate to previous physiological experiments. Isogeneic isolated islets were transplanted into diabetic Albino Oxford rats, resulting in normoglycaemia. After at least 5 wk, graft-receiving organs were removed and several antibodies were employed to detect insulin, neuron-specific proteins, and cholinergic and noradrenergic nerve fibers. Islets in all three receiving organs contained viable insulin-positive B-cells. Neuron-specific enolase (NSE) as well as the growth-associated protein B-50 was observed at all sites. The cholinergic marker choline acetyltransferase (ChAT) was localized in islets grafts at all sites, but with the lowest density in the spleen. Staining for the noradrenergic markers tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) was observed in islet grafts at all sites with the lowest density in grafts under the kidney capsule. All these neurochemical substances were most frequently observed in fibers associated with blood vessels, which may be the route along which nerves grow into the graft. It can be concluded that 1) islet grafts in the liver, in the spleen and under the kidney capsule become reinnervated; 2) the innervation pattern of the islet grafts differs only slightly from that in the control pancreatic islets; and 3) in combination with our previously physiological data, we can conclude that these nerve fibers are, at least partly, functionally active.**

☐ **Keywords — Islet transplantation; Reinnervation (innervation); Choline acetyltransferase; Tyrosine hydroxylase; Dopamine-β-hydroxylase; B-50 (GAP-43); Neuron-specific enolase.**

INTRODUCTION

Islets have been transplanted in diabetic rats at different sites (28,48) and with different volumes (49). Islet transplantation is sufficient to reestablish normoglycaemia due to sufficient insulin secretion by the implanted graft (48,49). However, due to the transplantation procedure, the islets lack the normal innervation by the autonomic nervous system. This may have consequences for an effective and fine-tuned regulation of insulin secretion. For example, during food intake, activation of the parasympathetic nervous system results in preabsorptive insulin release in normal controls, but is absent (44,46) or reduced in diabetic rats with islet grafts (48,49). Moreover, electrical stimulation of the dorsal vagus nerve to dog intrasplenic islet grafts did not result in an enhanced insulin secretion (23), which may indicate absence of parasympathetic innervation.

Stimulation of the sympathetic nervous system by electrical stimulation (13) or by exercise (18,19), on the other hand, showed inhibition of insulin secretion. This is similar to normal conditions in which the activation of the sympathetic nervous system inhibits insulin release via noradrenergic or peptidergic pathways (2–5,17,33). Other investigations, however, found that insulin secretion was not reduced during exercise after autotransplantation of islets in the dog spleen, suggesting denervation of the islets (40). In contrast, coordinate pulsatile insulin secretion was maintained (38). Although there are some controversial findings, it is possible that the inhibition of insulin secretion is the result of noradrenergic reinnerva-

tion of islets in the liver (13,18). So far there is some anatomical evidence for reinnervation of transplanted islets (15,25–27,35,36) but these results were not related to physiological studies.

Therefore, we investigated in rats 1) whether islet grafts in the liver, in the spleen, and under the kidney capsule become reinnervated with noradrenergic and cholinergic nerve fibers following the transplantation procedures used in our laboratory; 2) whether there is any difference in reinnervation patterns at these different sites; and 3) how the results relate to previous physiological experiments. We used immunocytochemical staining procedures to detect insulin. The neuronal markers neuron-specific enolase (NSE) (9) and the growth-associated protein B-50 [GAP-43] (32) were used to stain nerve fibers. The latter is also associated with the growth of these fibers and synaptic plasticity (12,14). Choline acetyltransferase (ChAT) was used to stain the cholinergic nerve fibers (47). For detection of noradrenergic nerve fibers we chose the general used marker tyrosine hydroxylase (TH) and the more specific marker dopamine-β-hydroxylase (DBH).

METHODS

Animals and Animal Care

Male inbred Albino Oxford (AO/G) rats, body weight ± 310 g, obtained from the Central Animal Laboratory of the University of Groningen, were used. Diabetes was induced by an intravenous injection of 70 mg/kg streptozotocin (Zanozar, Upjohn, Kalamazoo, MI). This resulted in loss of body weight, glucosuria, increased water intake, and blood glucose levels of more than 20 mmol/L. Two to three weeks after the diabetes induction, islet tissue isolated from isogeneic islet donors with a body weight of 350–400 g, was transplanted into the portal vein ($n = 7$), the spleen ($n = 7$), or under the kidney capsule ($n = 6$). The animals became normoglycaemic within 1 wk.

The animals were housed individually in perspex cages and they had free access to water and standard rat chow (Hope Farms, Woerden, The Netherlands). They were maintained on a 12:12 h light-dark cycle at a room temperature of about 20°C. At least 5 wk after transplantation, animals were sacrificed for histological investigation. Control animals ($n = 8$) were housed under the same conditions and were sacrificed for histological investigation of the pancreas.

Islet Isolation and Transplantation

The rat islet isolation method as used in our laboratory has been described previously (50). Briefly, the pancreas was distended by infusing 10 mL Kreb's Ringer solution containing 25 mM HEPES and 10% bovine serum albumin into the pancreatic duct. The pancreas was then excised and cut into small pieces with a pair of scissors. A two-stage collagenase (Sigma Type XI, 2200 U/mg, Sigma, St. Louis, MO) digestion was performed at 37°C at concentrations of 1.2 and 0.7 mg/mL, respectively. Islets were separated from the exocrine tissue using a discontinuous dextran gradient (Sigma industrial grade, MW 70.000). Further purification of the islets was obtained by handpicking to eliminate nonseparated lymph nodes, vascular, and ductal tissue from the islet grafts. Islets were identified with the aid of a dissection microscope (Bausch and Lomb 31-28-06) and a fluorescent lamp (Bausch and Lomb 31-33-66). With this illumination, rat islets appear as distinct ochreous bodies, whereas lymph nodes and exocrine tissue are grey. The reliability of this method has been confirmed by histology and dithizone staining.

The total islet volume obtained was determined by measuring the islet diameters, expressed as the mean of two axes, of islets in a 5% aliquot of the islet suspension. By assuming the islets to be perfect spheres, islet volume was calculated. Grafts of 5 µL endocrine tissue were prepared by taking an appropriate portion of the total islet suspension. This volume is about 50% of the content of a normal adult pancreas, as determined by measuring insulin content of a volume of 10 µL (48). Transplantation into the liver or spleen was performed immediately after the islet isolation by direct puncture with a 23 gauge butterfly needle in the portal vein or splenic parenchyma, respectively. During infusion into the spleen, the splenic pedicle was manually occluded to reduce possible islet loss to the liver (11). Transplantation under the kidney capsule was performed at the upper pole by carefully expelling the islets from a polyethylene tube introduced at the lower pole of the kidney. After the transplantation was completed, the syringes, butterfly needles and the polyethylene tubes were examined to confirm that all islets had been transplanted.

Immunocytochemical Procedure

Under pentobarbital anesthesia, animals were perfused with 50 mL heparinized saline followed by 300 mL 4% paraformaldehyde solution, containing 0.2% picric acid and 0.05% glutaraldehyde in 0.1 M phosphate-buffered saline (PBS). The pancreas, the liver, the spleen, or the kidney were removed and stored in 0.1 M PBS with sodium azide until the staining procedure.

The tissue was cryoprotected in a 30% buffered sucrose solution during 2 days. Thereafter the tissue was quickly frozen and slices of 20 µm were cut at a microtome at −20°C and immediately thaw-mounted on gelatin-coated slides. After drying and rinsing in 0.01 M

PBS, the slices were immersed in 0.1% H_2O_2, again rinsed in PBS, and preincubated with the appropriate normal sera (see below). Slices were subsequently incubated overnight at 4°C in [1] guinea-pig anti-porcine insulin (Dakopatts, Carpinteria, CA), diluted 1:200 in 0.01 M PBS and 0.5% triton X-100; [2] rabbit anti-tyrosine hydroxylase (TH, Eugene Tech International, Ridgefield Park, NJ), diluted 1:500 in PBS and 0.5% triton X-100; [3] rabbit anti-dopamine-β-hydroxylase (DBH, Eugene Tech International), diluted 1:500 in PBS and 0.5% triton X-100; [4] goat anti-choline acetyltransferase (ChAT, kindly donated by Dr. L.B. Hersch), diluted 1:1000 in PBS and 0.5% triton X-100; [5] rabbit anti-neuron specific enolase (NSE, Serva, Heidelberg, Germany), diluted 1:200 in PBS; and [6] mouse anti-B-50 [NM4] (kindly donated by Dr. M. Mercken and Innogenetics BV, Ghent, Belgium), diluted 1:10000 in PBS and 0.4% triton X-100.

After rinsing in PBS and preincubation in normal goat serum for staining nos. [1], [2], [3], and [5], in normal rabbit serum for [4] or normal sheep serum for [6], the sections were incubated 2 h at room temperature in the secondary antibodies goat anti-rabbit IgG for [1], [2], [3], and [5] (1:100, Zymed, San Francisco, CA), rabbit anti-goat IgG for [4] (1:100; Sigma), and biotinylated sheep anti-mouse IgG for [6] (1:200; Amersham, Little Chalfont, UK). After rinsing, the slices were incubated in rabbit peroxidase-anti-peroxidase (PAP) (1:500; Dakopatts) and goat PAP [1:500; Dakopatts)] for [1], [2], [3], [5], and [4], respectively, or in Streptavadin-HRP (1:200; Zymed) for [6]. Double bridging was used for [2], [3], and [4] to obtain a more intense staining. Finally, the diaminobenzidine(DAB)-H_2O_2 reaction (30 mg DAB and 0.01% H_2O_2/100 mL TRIS buffer) was used to stain the slices.

After immunocytochemical staining, sections were dehydrated, cleared in xylene, coverslipped, and examined by use of a standard light microscope. The islets were divided in categories dependent on whether the estimated number of insulin-positive cells was more or less than 40%. The presence of immunoreactive fibers in the other slices was assessed and semiquantified. The results are presented in Table 1. Control experiments were performed by omitting the primary antibody and revealed immunonegative results.

RESULTS

Pancreas

The pancreas of normal control rats contained viable islets of Langerhans, as identified by immunocytochemistry for insulin. All islets contained predominantly insulin-positive cells (Fig 1A). Labelling of the neuronal marker NSE was observed in both the exocrine and the

Table 1. Presence of immunoreactive insulin and immunoreactive nerve fibers in the pancreas and islet grafts in the implantation organs

	Insulin	NSE	B-50	ChAT	TH	DBH
Pancreas	++	+++	+++	++	++	++
Liver	++	++	+++	+	++	++
Spleen	+	+	+++	+/–	++	+
Kidney Capsule	++	++	++	+	+	+/–

+/–, occasional fibers; +, some islets with few B-cells; low number of fibers; ++, islets with many B-cells; moderate number of fibers; +++, high number of fibers.

endocrine part of the pancreas. NSE-positive nerve fibers were most prominent at the mantle of the islets of Langerhans (Fig 2A). Staining of the growth-associated protein B-50 was very clear. The exocrine part of the pancreas contained many thick nerve fibers, sometimes including varicosities. In contrast, the islets (Fig. 3A) were mainly characterized by B-50 positive varicose-like structures. ChAT-immunoreactivity (Fig. 4A) was mainly observed at the periphery of these islets, and sometimes in the core of the islets. Cholinergic ganglion cells were found in the exocrine part of the pancreas (not shown in figure). In the islets of Langerhans, TH-positive nerve fibers (Fig. 5A) were observed around blood vessels, but also in the islet tissue itself. The exocrine pancreas contained more and clearer TH-positive nerve fibers than the endocrine tissue. In both exocrine and endocrine part of the pancreas, nerve fibers contained varicosities. Reaction with the DBH-antibody (Fig. 6A) showed similar results, though less clear fibers and more varicosities were observed in the islet tissue than after staining for TH. The results of the immunocytochemical stainings are summarized in Table 1.

Liver

Islets in the liver (Table 1) were identified by immunocytochemistry for insulin. Approximately 90% of the viable transplanted islets contained more than 40% insulin-positive cells (Fig. 1B). In general these islets were situated within the vasculature and contained many B-cells. The neuronal marker NSE was present in the liver parenchyma and also in the transplanted islets. However, when compared to islets in the normal pancreas, the NSE staining was less pronounced (Fig 2B). Immunoreactivity for the growth-associated protein B-50 was clearly visible within the islets (Fig. 3B) and characterized by many terminals. This is in contrast to the surrounding liver parenchyme, which contained hardly any positive B-50 staining. Only the vasculature within the liver contained clear B-50 positive nerve fibers. ChAT-immunoreactivity was observed in many transplanted islets, but the density was low in most islets. Occasionally
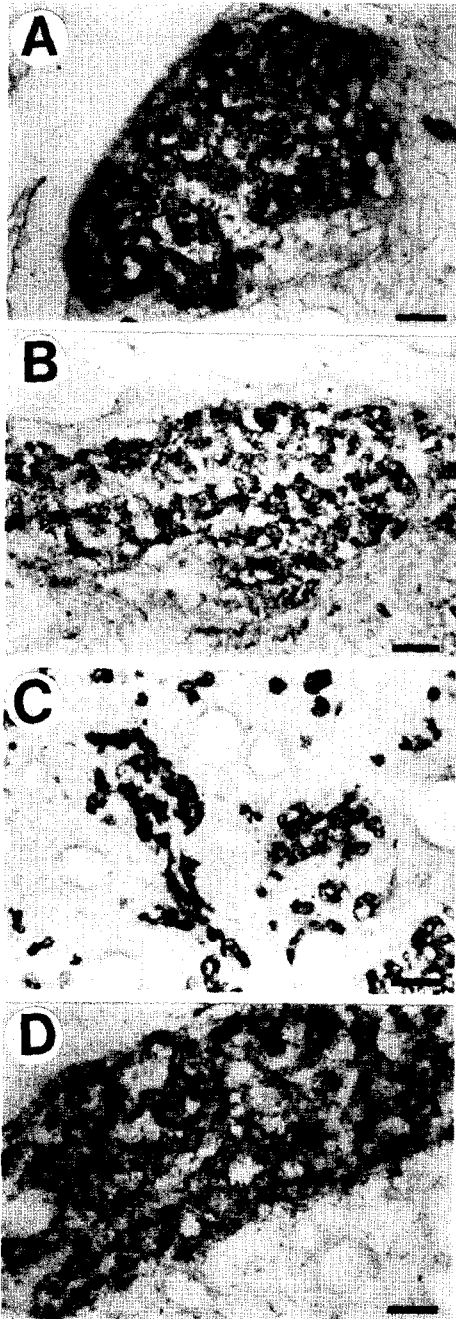
Fig. 1. (A–D) Photomicrographs of insulin immunoreactivity in the rat pancreatic islet (A) and in islet grafts in the liver (B), the spleen (C), and under the kidney capsule (D). Most cells were insulin-positive in islets in the pancreas, liver, and under the kidney capsule, whereas some islets in the spleen also contained many insulin-negative cells as shown in this (C). Scale bar = 50 μm.
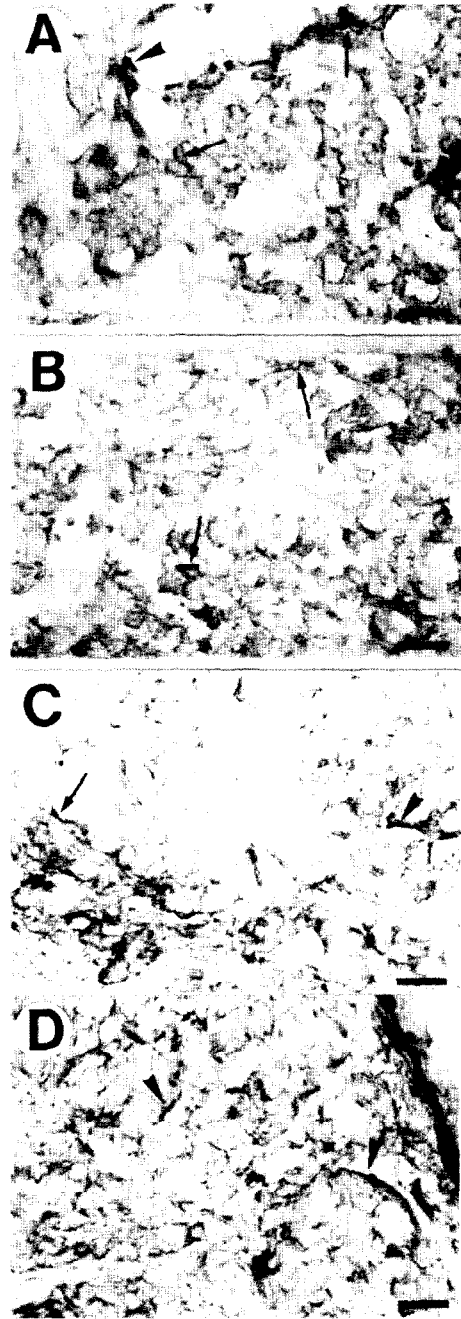

Fig. 2. (A–D) Photomicrographs of the immunoreactivity of the neuronal marker NSE in the rat pancreatic islet (A) and in islet grafts in the liver (B), the spleen (C), and under the kidney capsule (D). NSE-positive fibers were mainly observed at the mantle of the islets, but also amidst the islets (arrows) (A, B). Association with the vasculature was freqently observed (arrow head) (A). NSE-positive fibers in islets in the spleen were mainly observed in islets situated in blood vessels, and predominantly at the islet mantle (arrow) and vasculature (arrow head) (C). The antibody to NSE mainly stained vasculature-associated nerve fibers in islet grafts under the kidney capsule (arrow heads) (D). Scale bar = 25 μm.

islets contained a denser immunoreactivity for ChAT. The enzyme was predominantly present around blood vessels in these islets (Fig. 4B). TH and DBH positive nerve fibers were predominantly associated with the vas-
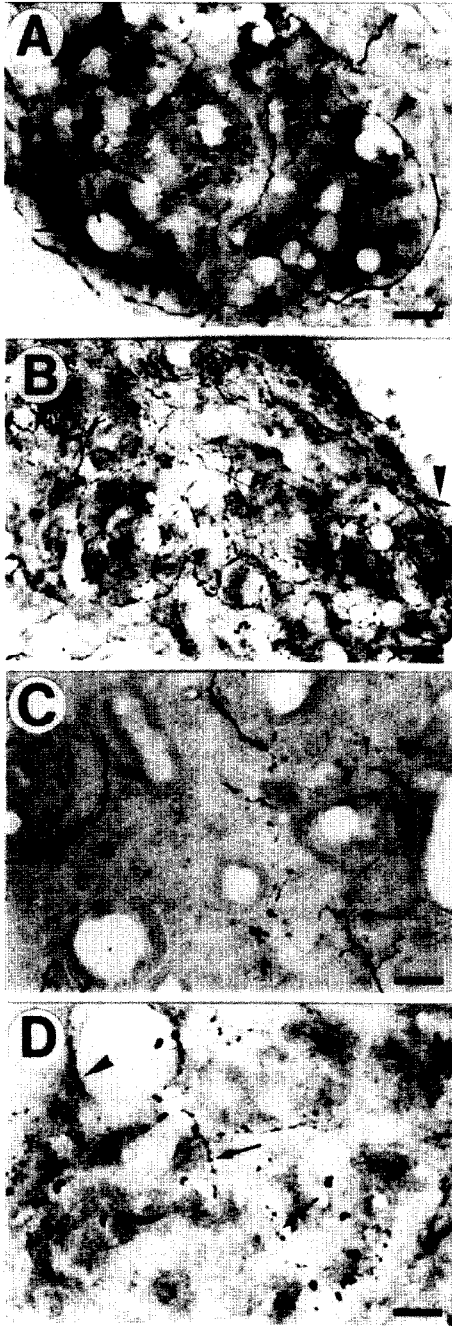
Fig. 3. (A–D) Photomicrographs of the immunoreactivity of the neuronal marker B-50 in the rat pancreatic islet (A) and in islet grafts in the liver (B), the spleen (C), and under the kidney capsule (D). (A) Thick B-50 positive nerve fibers run along the vasculature in the pancreas and around the islets (arrow head), whereas the islets are mostly characterized by varicosities (arrow). B-50 positive nerve fibers are only observed within the liver vasculature (arrow head), and are more frequent and more varicose-like in the transplanted islets (arrows) (B). The splenic pulp contains B-50 positive nerve fibers, but the immunoreactivity is denser in the islet grafts. This photomicrograph shows very thick B-50-positive fibers (arrow head) (C). These thick nerve fibers are observed in islets, which contained many insulin-negative cells (see Fig. 1C). The arrows point at more varicose-like nerve fibers in the intrasplenic islet grafts. Density of B-50-positive nerve fibers in islet grafts under the kidney capsule (D) is lower than at the other sites, with fibers associated with a blood vessel (arrow head) and with islet cells (arrows). Scale bar = 25 μm.
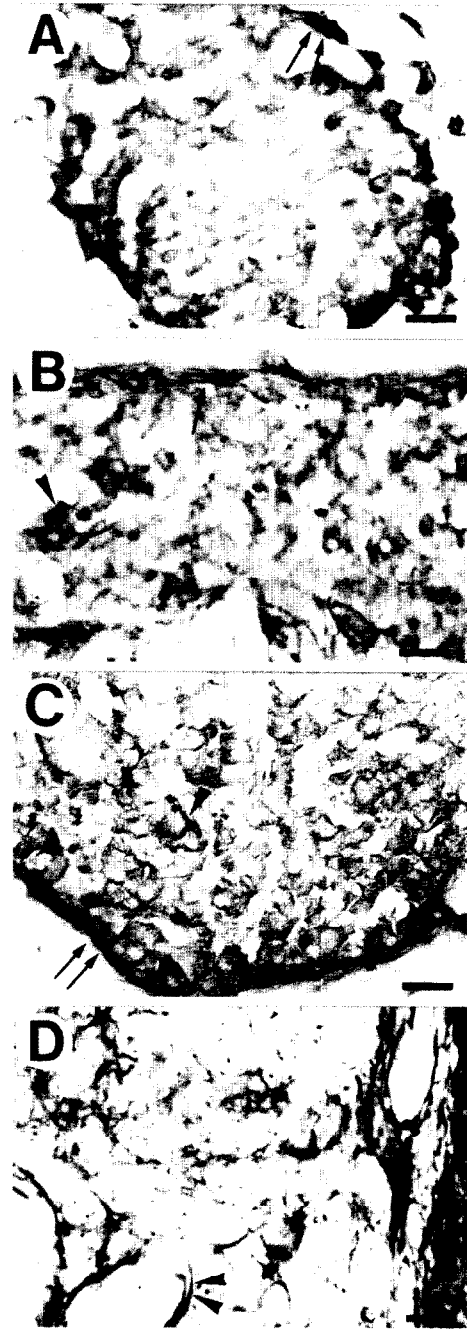

Fig. 4. (A–D) Photomicrographs of ChAT immunoreactivity in the rat pancreatic islet (A) and in islet grafts in the liver (B), the spleen (C), and under the kidney capsule (D). The cholinergic marker ChAT was predominantly observed at the mantle of the islets (arrows) or associated with the vasculature in the islet grafts (arrow heads). ChAT-immunoreactivity in islets in the spleen was only observed in islets situated in blood vessels. Scale bar = 25 μm.
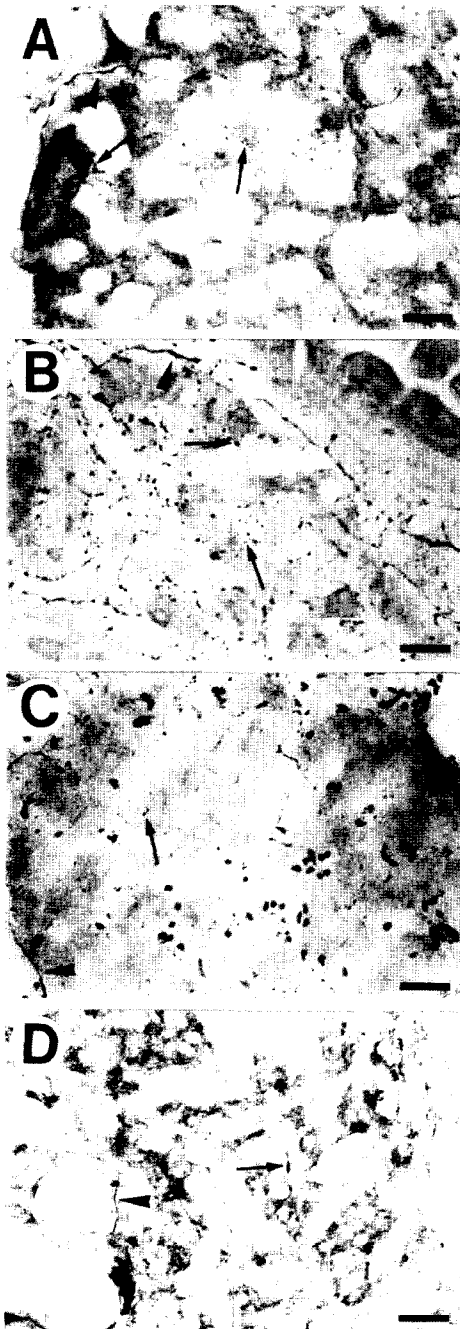
Fig. 5. (A–D, left column) Photomicrographs of TH-immunoreactive nerve fibers in the rat pancreatic islet (A) and in islet grafts in the liver (B), the spleen (C), and under the kidney capsule (D). TH-positive nerve fibers were clearly visible in islets in the pancreas, liver, spleen, and under the kidney capsule and contained varicose structures (arrows). The noradrenergic markers were most regularly observed in association with blood vessels and in association with (ingrowing) capillaries in the islets or around the islets (arrow heads). Scale bar = 25 µm.
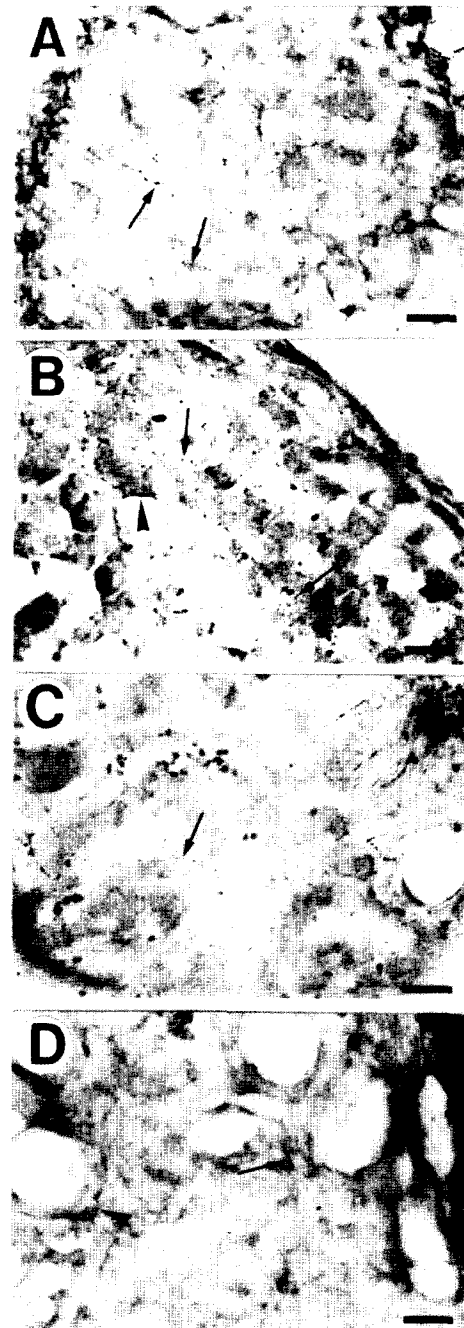
Fig. 6. (A–D, right column) Photomicrographs of DBH-immunoreactive nerve fibers in the rat pancreatic islet (A) and in islet grafts in the liver (B), the spleen (C), and under the kidney capsule (D). DBH-positive nerve fibers were associated with the vasculature (arrow heads in B and D). Varicose-like nerve fibers (arrows) were observed within the islets in the pancreas, in the liver, the spleen, and under the kidney capsule (arrows). Islet grafts in the latter site contained only an occasional DBH-positive nerve fiber. Scale bar = 25 µm.

culature in liver tissue. Both the markers TH (Fig. 5B) and DBH (Fig. 6B) were observed around blood vessels in the grafts, but also amid islet cells. The nerve fibers in the grafted tissue were less prominent as the nerve fibers in the liver and, moreover, were more varicose-like.

Spleen

Islets grafted to the spleen were observed in the vasculature and trabeculae and within the splenic pulp. Nearly half of the islets contained less than 40% insulin-positive cells which were located in the central core of the islets (Fig. 1C). This suggests that there were only few B-cells and many other cell types. Immunocytochemistry for NSE resulted in staining of fibers in the main blood vessels in the spleen. Islets that were situated in these blood vessels showed some positive staining for NSE (Fig. 2C). This staining was mainly associated with the microvasculature in the islets and hardly with the islet cells. The staining for B-50 in the spleen resulted in clearly visible nerve fibers along the blood vessels and also in the islet grafts (Fig. 3C). Many varicose-like structures were observed within the islets. Nerve fibers in the major splenic blood vessels stained positively for ChAT. Some islets were localized in these blood vessels and ChAT-immunopositive nerve fibers were mainly observed in the small blood vessels in these islets (Fig. 4C). Nerve fibers were rarely observed in the vicinity of islet cells. In other islets, which were only surrounded by splenic pulp, ChAT-positive nerve fibers were not observed. The splenic trabeculae and most prominent blood vessels were densely innervated with TH-positive nerve fibers characterized by varicosities. This was similar for DBH-positive nerve fibers, although these fibers were less clear. In some cases noradrenergic nerve fibers within the islets (TH: Fig. 3C; DBH: Fig. 4C) were found to originate from sympathetic nerves associated with the blood vessels. A summary of the above-mentioned results is provided in Table 1.

Kidney Capsule

Unlike the islet grafts in the liver and the spleen, the islets under the kidney capsule were not only characterized as single islets, but also as clusters of islets separated by connective tissue (Fig. 1D). Most islet cells were viable and contained insulin (Table 1). Immunocytochemistry for NSE (Fig. 2D) resulted in stained fibers related to the vasculature within the graft, in a similar fashion as observed for the other implantation sites. The growth-associated protein B-50 was present in the islet grafts (Fig. 3D); however, the staining was somewhat less dense compared to the other sites. ChAT-positive fibers were also predominantly localized around blood vessels, but were also observed amidst islet cells (Fig.

4D). These nerve fibers appeared to originate from the capsule. The presence of TH (Fig. 5D) and DBH (Fig. 6D) containing nerve fibers in the islets under the kidney capsule was low or occasionally observed, respectively. These fibers seemed to originate from axons associated with ingrowing blood vessels. TH-positive nerve fibers sometimes contained varicosities.

DISCUSSION

This study demonstrates the presence of insulin, neuronal markers, and neurotransmitter synthesizing enzymes in pancreatic islets and isogeneic islet grafts in the liver, in the spleen, and under the kidney capsule in rats. Numerous B-cells were found in the pancreatic islets, which contained a moderate to dense innervation for NSE, B-50, ChAT, TH, and DBH. ChAT and NSE-positive nerve fibers in pancreatic islets are mainly observed in the mantle of the islets, as previously described for ChAT (47). The exocrine pancreas contains thick TH, DBH, and B-50 positive nerve fibers associated with capillaries. Fibers within the islets are more characterized by varicose structures, which looked similar to those observed after staining for adrenergic (4) and peptidergic (10) fibers in the endocrine pancreas. All animals provided with an islet graft became normoglycaemic. Moreover, glucose tolerance in previously performed glucose infusion tests and meal tests was similar in animals with islet grafts at these sites (49,50). However, some of the grafted islets in the spleen contained only few B-cells. It appeared that these B-cells were larger than those in islet grafts with a normal density of B-cells. The reason for this phenomenon remains unclear, but one might suggest that these B-cells were very active, which is accompanied by a certain degree of hypertrophy. The innervation pattern, as observed in the islet grafts, differed slightly from that in the control pancreatic islets. TH and DBH-positive fibers in the grafts were less clear in the islet grafts, in particular under the kidney capsule. In the grafts, all types of fibers were observed around blood vessels within the grafted islets. The distribution amidst islet cells was less clear.

The presence of NSE (9) and B-50 (14,32,42) in the islets in the pancreas and in the islet grafts indicate that nerve fibers are present. The presence of B-50 positive nerve fibers, in particular nerve endings, in the islet grafts suggest growth and regeneration of the nerve fibers. In addition, immunoreactivity for the growth-associated protein B-50 in the pancreas could imply a continual remodelling of nerve fibers in the pancreas (12), which is in accordance with the regenerative capacity of pancreatic islets (34) as also reported for the enteric nervous system (41). B-50-positive nerves in the

pancreatic islets appeared to be more associated with noradrenergic than cholinergic nerve fibers. Immunofluorescence double labeling (unpublished observations) confirmed these observations, although some ChAT-positive fibers in exocrine pancreatic tissue were B-50-positive. Little is known about the factors responsible for the ingrowth of peripheral nerve fibers into the transplanted islets or organs. Target cells might secrete factors affecting their own innervation pattern (16), as reported for muscle cells (8). With regard to islet cells, it might be suggested that the secretion of insulin or insulin-like growth factor can act as a trophic factor for ingrowth of new nerve fibers into the graft (20,21,33). It is previously suggested that reinnervation occurs in association with revascularisation (15,39), the latter being complete after the first or second week posttransplantation (15,29,30). New endothelial cells were found to originate from the graft-receiving organ (6,29), and probably new nerve fibers also originate from the implantation organ (35). In our studies there is indeed a strong association of the nerves with blood vessels, which may be the route along which nerves grow into the graft.

ChAT-immunoreactivity was found in islet grafts in the liver, under the kidney capsule, and only rarely in the spleen. In other studies, acetylcholinesterase-positive nerve fibers were observed in islet grafts in the rat liver (13,35,36), the mouse liver, and under the mouse kidney capsule (25,26). However, it has been shown in the pancreas that acetylcholinesterase is not entirely specific for cholinergic neurons and that ChAT is a more reliable cholinergic marker than acetylcholinesterase (47). Staining for vasoactive intestinal peptide (VIP) as an alternative parasympathetic marker (5) showed labelling in islet grafts under the kidney capsule in rats and mice (25,26,39), but not in the liver in mice (26). VIP immunoreactivity in the kidney itself, however, was shown to be at least partly sympathetic (24). In the spleen, there were no previous indications for cholinergic innervation of islet grafts. Moreover, evidence of cholinergic innervation of the spleen itself is unclear, because staining for acetylcholinesterase was related to noradrenergic nerves and the reported low activity of choline acetyltransferase appeared not to be of vagal origin (7). Therefore the parasympathetic influences in the spleen and thus also in the intrasplenic islet grafts are presumably absent, or limited as best, whereas these influences are present in islet grafts in the liver and under the kidney capsule.

Parasympathetic nerve fibers are considered to be stimulated during the preabsorptive phase of a meal and thereby increase insulin release from the B-cells of the islets of Langerhans (43). During meal tests in islet-grafted rats, the preabsorptive phase of the insulin release is not (44,46), or only partly, enhanced (48,49). These reductions in insulin responses were considered to be the effect of the denervation of the islet grafts due to the transplantation procedure, because the grafted volume appeared to be sufficient (49). The present and above-mentioned studies, however, give indications that islet grafts also become reinnervated with parasympathetic nerve fibers, although parasympathetic influences in the spleen remain unclear (7). Because after transplantation the ChAT-positive nerve fibers appear to be less dense than the noradrenergic nerve fibers, and because the preabsorptive insulin response is smaller than in controls, it is possible that the function of the observed nerve fibers is not satisfactory for adequate insulin secretion.

Our study in rats showed ingrowth of noradrenergic nerve fibers into transplanted islets in the liver, in the spleen, or under the kidney capsule. Similar results were also reported for isogeneic islet transplantations to these sites in mice (25,26), into the liver after isogeneic (13,15), or allogeneic transplantation (35,36) and after autotransplantation of pancreatic tissue into the anterior eye-chamber (1). Noradrenergic nerve fibers were absent after syngeneic islet transplantation under the kidney capsule in rats (39), which is in contrast to our observations and those of Korsgren (25,26). Synaptic-like contacts with islets were found in an electron microscopic study from 4 wk after autotransplantation of pancreatic fragments in the spleen of the dog (27). After transplantation of islets in the portal vein of diabetic rats, active ingrowth of nerve fibers from the portal area into the islet graft was observed beyond the second week after transplantation. These nerve fibers were not found to be in direct contact with the transplanted islet cells in the liver, but rather with the capillaries within the islets (15). Nerve endings were also reported to be in close vicinity of A-cells and B-cells (35). Although several negative observations exist, the majority, including our study, indicate that noradrenergic innervation of the grafts exists. The physiological functionality of noradrenergic reinnervation has been proven to be sufficient for the liver (13,18,38) and renal subcapsular space (19) as implantation site.

In our previous physiological experiments, we showed that insulin secretion was reduced during exercise, even after adrenodemedullation (18). We suggested that noradrenergic reinnervation of intrahepatic islet grafts was the causal factor (18), which is in accordance with the results reported in the present study. The reduced insulin secretion may be caused by a reduced islet blood flow (22). Because glucagon levels did not change after hepatic sympathetic nerve stimulation in animals with portal islet grafts, whereas insulin levels decreased (13), this option might not be valid. A second option is that locally released norepinephrine or co-released peptides act through paracrine mechanisms on the B-cells, as also reported for islets in the pancreas in situ (45,51), because

the intra-islet "core-to-mantle" capillary perfusion system is reestablished (30,31). In this option, reinnervation combined with a higher sensitivity of adrenergic receptors on the transplanted islets (37) may inhibit insulin secretion.

In conclusion, the present study provides anatomical evidence for ingrowth of noradrenergic and cholinergic nerve fibers in pancreatic islet grafts in the liver, the spleen, and under the kidney capsule. The noradrenergic nerve fibers may be functional in suppression of insulin secretion during activation of the sympathetic nervous system as shown in previous exercise experiments. Although the parasympathetic control may be impaired due to a less adequate function of a lower number of fibers, the anatomical signs of reinnervation of islet grafts appear to have, at least in part, functional importance.

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