Crossover Study on Effects of Duct Obliteration, Celiac Denervation, and Autotransplantation on Glucose- and Meal-Stimulated Insulin, Glucagon, and Pancreatic Polypeptide Levels

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In segmental-pancreas transplantation the body and tail of the pancreas are used. In an experimental study in dogs, the effects of sequentially conducted removal of the right pancreatic lobe (pancreatic head), duct obliteration, celiac denervation, and autotransplantation were studied according to a crossover design. Two groups of dogs were studied. In both groups the right lobe of the pancreas was removed at primary operation, and the duct of the transected left lobe (body and tail) was injected with fibrin sealant. The left lobe was completely freed from surrounding tissue (celiac denervation) in group 1 (n = 9), and the innervation of the left lobe was left intact in group 2 (n = 8). At 12 wk, two dogs in group 1 and four dogs in group 2 underwent successful autotransplantation of the left lobe. Pancreatic hormone secretion was stimulated by intravenous glucose injection and test-meal administration before primary operation and at 11 and 18 wk thereafter. The combination of removal of the right lobe and duct obliteration led to a decrease in glucose tolerance at both stimulation tests and a decrease in peripheral insulin release after intravenous glucose injection. At test-meal administration, no change in insulin and glucagon levels was demonstrated. If celiac denervation was added, similar results were obtained based on the understanding that the peripheral insulin release after the test meal was significantly elevated. Meal-stimulated pancreatic polypeptide response was abolished in both groups. Removal of the right lobe leads to parasympathic denervation of the left lobe, and celiac denervation mainly interferes with α-adrenergic innervation. Cholecystokinin was probably responsible for the absence of a decrease in insulin secretion at test-meal administration after primary operation. Secondary autotransplantation led to elevation of peripheral insulin release at both tests but had no demonstrable influence on glucose tolerance expressed in K values.
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Much experimental work has been published to show that duct obliteration interferes with endocrine function either after in situ duct obliteration or after segmental duct-obliterated autotransplantation. In most of the studies published, duct obliteration, denervation, and autotransplantation were performed in groups of dogs that were subjected to a combination of two or more of these manipulations. In this study, we tried to unravel the effects of denervation, duct obliteration, and autotransplantation to gain more insight into endocrine function after pancreas transplantation.

MATERIALS AND METHODS

The experiments were performed in inbred beagles weighing 9–15 kg. The dogs were obtained from the Central Institute for the Breeding of Laboratory Animals (Zeist, The Netherlands).

Two groups of dogs were studied. In both groups the right lobe of the pancreas was removed at the primary operation, and the pancreatic duct of the left lobe was injected with a fibrin sealant (Tissucol, Immuno Chemie, Paris). In group 1 (n = 9) the innervation to the left lobe, originating from the celiac and superior mesenteric ganglia, was cut by dissecting the left lobe free from its surrounding tissue except for the splenic artery and vein, which were stripped bare over ~1 cm. In group 2 (n = 8) the innervation to the left lobe was left intact. After the operation, the dogs were allowed to eat their regular diet to which protease-amylase-lipase granules (Pancreasgranulaat, Organon, Oss, The Netherlands) were added for exocrine substitution. Twelve weeks after primary operation, six dogs, four from group 1 and two from group 2, underwent delayed segmental auto-

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transplantation of the previously obliterated left lobe. Anesthesia at both primary and secondary operation was induced with thiopental sodium (Nesdonal, Rhône-Poulenc, France) 25 mg/kg body wt i.v., and the dogs were kept on spontaneous respiration with nitrous oxide oxygen and halothane, 1.25–1.50%.

For the analysis of the effect of operation on pancreatic hormone secretion, intravenous glucose tolerance tests (IVGTTs) were performed, and a test meal (TM) was administered before primary operation, 12 wk postoperation, and 6 wk after delayed segmental-pancreas autotransplantation. At IVGTT, a bolus of 0.5 g/kg i.v. glucose was injected, and glucose and insulin concentrations in peripheral blood were determined up to 1 h after injection. TM consisted of a semiliquid mixed meal with 45% carbohydrate, 30% protein, and 24% fat. Before and up to 5 h after completion of the TM, peripheral blood samples were drawn for the determination of glucose, insulin, glucagon, and pancreatic polypeptide (PP) determination. Samples for PP were collected only before and 60 min after completion of the TM. Results of IVGTT were expressed in K values (means ± SE) and incremental insulin levels of the area under the curve (means ± SE) for the test period ($\Delta AUC_{insulin}$). Results of TM for glucose, insulin, and glucagon were expressed in $\Delta AUC_{glucose}$, $\Delta AUC_{insulin}$, and $\Delta AUC_{glucagon}$, whereas PP stimulation was described in ΔPP, indicating $PP(t_{60}) - PP(t_0)$. Student's t test was used for statistical analysis of the results.

RESULTS

All dogs survived the primary operation and retained their preoperative body weight. Delayed segmental-pancreas autotransplantation could only be successfully performed in six dogs because the operation turned out to be technically extremely difficult.

The effects of primary operation on endocrine function are listed in Table 1. No difference in the effect of operation on IVGTT between groups 1 and 2 was found; in group 1, K values dropped from 3.5 ± 0.2 preoperation to 1.6 ± 0.3 at 12 wk, and in group 2 they dropped from 3.4 ± 0.3 to 1.6 ± 0.1. The effect of operation on $\Delta AUC_{insulin}$ at IVGTT was also similar in both groups (from 1744 ± 304 to 953 ± 158 min · μU · ml^{-1} in group 1 and from 1488 ± 235 to 744 ± 176 min · μU · ml^{-1} in group 2). After TM administration, however, significant differences between the two groups were observed. In group 1 an increase in $\Delta AUC_{insulin}$ from 120 ± 22 to 274 ± 64 h · μU · ml^{-1} at 12 wk was observed, and in group 2 no significant change was found. The corresponding increase in $\Delta AUC_{glucose}$ amounted to 0.6 ± 0.2 to 6.6 ± 0.7 h · mM in group 1 and from 0.7 ± 0.2 to 6.1 ± 0.3 h · mM in group 2. No significant changes in $\Delta AUC_{glucagon}$ could be demonstrated for both groups, but the PP levels declined from 604 ± 135 and 363 ± 39 pM preoperation to 8 ± 2 and 9 ± 4 pM in groups 1 and 2, respectively, at 12 wk.

Delayed segmental autotransplantation induced a significant increase in peripheral insulin levels both at IVGTT and after TM administration. The mean difference between pre- and posttransplantation $\Delta AUC_{insulin}$ levels at IVGTT was 1352 ± 416 μU and was 188 ± 51 μU after TM, with posttransplantation $\Delta AUC_{insulin}$ levels of 2205 ± 358 μU at IVGTT ($P < .01$) and 406 ± 41 μU at TM administration ($P < .02$). The mean difference between pre- and posttransplantation K values was 0.1 ± 0.1, with posttransplantation K values of 1.6 ± 0.2.

DISCUSSION

The experiment can be separated into two parts. The first part focused on the effects of duct obliteration, removal of the right lobe, and denervation. In the second part the effect of transplantation of a previously duct-obliterated left lobe was analyzed. After primary operation both at IVGTT and at TM administration, a decrease in glucose tolerance was observed, and this decrease was accompanied by hypoinsulinemia at IVGTT in both groups. This result is in contrast to the observed normoinsulinemia in group 2 and hyperinsulinemia in group 1 at TM administration. Apparently, TM more effectively stimulates insulin release, a stimulus that seems to be even more effective if celiac denervation has also been performed. Because α-adrenergic stimulation leads to a decrease in insulin release, interference with these nerve pathways seems to be the main effect of celiac denervation. The almost complete abolishment of PP stimulation after removal of the right lobe supports our earlier findings (1) and those of Tiscornia et al. (2) indicating that the right lobe is the main route of entry for parasympathetic fibers originating from the nerve plexus localized along the duodenum.

The finding that a postoperative decrease in glucose tolerance is found with hypoinsulinemia at IVGTT and normo-

TABLE 1
Effect of removal of the right lobe and duct obliteration of the left lobe with (group 1, $n = 9$) or without (group 2, $n = 8$) celiac denervation on glucose and hormone response patterns after intravenous glucose and test-meal stimulation

	Preoperation		12 wk postoperation	
	Group 1	Group 2	Group 1	Group 2
Intravenous glucose tolerance test				
K	3.5 ± 0.2	3.4 ± 0.3	1.6 ± 0.3*	1.6 ± 0.1*
$\Delta AUC_{insulin}$ (μU)	1744 ± 304	1488 ± 235	953 ± 158*	744 ± 176*
Test meal				
$\Delta AUC_{glucose}$ (mM)	0.6 ± 0.2	0.7 ± 0.2	6.6 ± 0.7†	6.1 ± 0.3†
$\Delta AUC_{insulin}$ (μU)	120 ± 22	132 ± 37	274 ± 64‡	188 ± 46
$\Delta AUC_{glucagon}$ (pg)	334 ± 51	311 ± 54	248 ± 34§	307 ± 52§
ΔPP (pM)	604 ± 135	363 ± 39	8 ± 2	9 ± 4

Values are means ± SE. AUC, area under the curve.
*$P < .001$, †$P < .05$, ‡$P < .03$, §NS vs. preoperation.

or hyperinsulinemia after TM can be explained by an insulinotropic effect of some enteropancreatic stimulation mechanism activated by intraluminal administration of TM. Protein and fat are well-known stimulants of cholecystokinin release, and the well-documented insulinotropic effect of cholecystokinin can explain the normoinsulinemia (group 2) and hyperinsulinemia (group 1) at TM administration (3). The reason why these serum levels of insulin are incapable of inducing euglycemia, however, needs further explanation. Perhaps the duct-obliteration–induced histological changes interfere with a qualitatively normal pattern of insulin delivery; recently, emphasis has been given to a role for an autonomously functioning neuronal network to coordinate secretory activity of pancreatic islets (4). This intact intrapancreatic neuronal network is claimed to be mandatory for pulsatile insulin delivery in the normally functioning pancreas. In the absence of pulsatile insulin release, much higher insulin levels are required to induce normoglycemia. Duct obliteration may have damaged this intrinsic network, because islet architecture is completely disrupted after duct obliteration (5), and the normoinsulinemia found 12 wk after duct obliteration in group 2 may have been incapable of inducing normoglycemia because of the fact that the histological changes interfere with normal pulsatile insulin delivery.

In the second phase of the experiment, we showed that segmental autotransplantation leads to elevation of stimulated peripheral insulin levels both at IVGTT and at TM administration. These higher peripheral insulin levels, as a result of bypassing the liver on first circulation, had no bearing on glucose tolerance expressed in *K* values, which is in contrast to what we have concluded from previous experiments (6). In those experiments comparing *K* values of in situ duct-obliterated left lobes, we found higher *K* values after segmental duct-obliterated autotransplantation. These were observations from two groups of dogs, whereas in this study, in situ duct obliteration and autotransplantation were sequentially conducted, and the autografted dogs served as their own controls. Based on the *K* values observed, we conclude that there is no advantage for the pancreas graft to drain to the portal system instead of draining to the systemic circulation.

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