Pancreatic islet transplantation: studies on the technique and efficacy of islet isolation and transplantation

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5 Contribution of partial pancreatectomy, systemic hormone delivery, and duct obliteration to glucose regulation in canine pancreas

Importance in pancreas transplantation*

Introduction

The results of clinical pancreas transplantation have improved considerably in the last decade in terms of 1- and 2-yr patient and graft survival [1]. For many reasons, the quality of metabolic control is not optimally documented. The tests performed give only a global insight into the endocrine performance of the graft and the reserve capacity. The mutual influence of donor and recipient pancreases cannot be unraveled and the side effects of immunosuppressive agents on glucose regulation cannot be studied in depth in a clinical setting. The studies published on the effect of pancreas transplantation on secondary diabetic complications are contradictory [2-4], either because secondary complications are already too advanced at transplantation or because of suboptimal graft function.

Important issues for determining the best way to expand the role of pancreas transplantation in the treatment of diabetic patients are whole versus segmental pancreas transplantation, systemic versus portal drainage of the graft's venous effluent, and management of exocrine secretion. The isolated

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contribution of partial pancreatectomy, denervation, systemic hormone delivery, or obliteration-induced histological changes and interruption of the enteropancreatic axis to glucose regulation after pancreas transplantation is essentially unknown. We studied these different aspects of pancreas transplantation in a crossover design in beagles by the consecutive performance of partial pancreatectomy, diversion of pancreatic hormone delivery from portal to systemic circulation, and duct obliteration, concentrating on changes in response patterns of the islet hormones insulin, glucagon and pancreatic polypeptide (PP) and on the role of cholecystokinin (CCK). The gut hormone CCK was studied because CCK not only influences gallbladder contraction and pancreatic exocrine function but also plays an important role in the regulation of postprandial pancreatic hormone release. Two different stimuli, intravenous glucose bolus injection and a test meal, were administered to study glucose regulation. Because partial pancreatectomy and duct obliteration may affect innervation of the endocrine pancreas, and bombesin-stimulated PP release seems largely neurally mediated [5-9], we also studied intravenous bombesin-stimulated PP release.

Research design and methods

Animals

Experiments were performed in inbred beagles weighing 9-15 kg (Central Institute for the Breeding of Laboratory Animals, Zeist, The Netherlands). They were maintained on a regular diet of semiliquid dog food (Complete Dog Food D-B, Hope Farms, Woerden, The Netherlands). After pancreatic duct obliteration the diet was supplemented with ~2 g/day protease-lipase-amylase granules (Pancreas Granulaat, Organon, Oss, The Netherlands) for exocrine substitution. General anesthesia for all operative procedures was induced with intravenous thiopental sodium (Nesdonal, Rhône-Poulenc, Paris, 25 mg/kg body wt), and maintained with an N_2O/O_2 (1:1)-halothane (1-2%) mixture after intubation. Atropine (0.05 mg/kg i.m.) was administered for premedication.

Experimental protocol

Glucose regulation was studied in two groups of dogs. Group 1 dogs (n = 14) were healthy unmodified, controls. Group 2 dogs (experimental dogs, n = 6) were studied at three intervals in a crossover design. The first function tests were performed 6 wk after partial (~70%) pancreatectomy, including body, tail, and part of the uncinate process of the pancreas with ligation of the inferior

mesenteric vessels, leaving regular enteric exocrine drainage from the duodenal pancreatic remnant intact (interval 1). Estimates of the weight of residual tissue are based on the weight of resected tissue and the mean weight of whole pancreases as obtained from previous experiments. After the function tests were completed, the cranial pancreaticoduodenal vein was transected at the junction with the portal vein, and an end-to-side anastomosis between the pancreaticoduodenal and the inferior caval vein was fashioned, thus shunting the remnant pancreas venous effluent from portal to systemic circulation. Two weeks after venous transposition, the function tests were repeated (interval 2). Finally, the ductal system of the pancreatic remnant was obliterated by injection through the ventral main pancreatic duct with 0.2–0.5 ml of the synthetic latex polymer neoprene (Neoprene Latex 671, Dupont de Nemours, Wilmington, DE) and again 6 wk after duct-obliteration function tests were performed (interval 3).

Function tests

Glucose regulation was studied by determining *1*) peripheral (jugular vein) plasma levels of glucose, insulin, glucagon, CCK and PP in dogs that had been fasted 18 h overnight and after meal stimulation (regular semiliquid meals consumed within 15 min that contained 45% carbohydrate, 24% fat and 30% protein); *2*) plasma glucose and insulin levels after intravenous glucose stimulation (intravenous glucose tolerance test [IVGTT]; 0.5 g/kg i.v. glucose) according to a method described previously [10]; and *3*) plasma PP levels after an intravenous bolus bombesin injection (12.5 ng/kg body wt; bombesin-14, UCB, Brussels).

Fasting hormone levels were expressed as the mean of values on 2 consecutive days. IVGTTs were used to determine both the glucose tolerance and glucose-stimulated insulin response. Glucose tolerance was expressed in K_g values (percent decline of glucose level per minute, calculated from 10 min untill the resumption of basal values). The insulin response at IVGTT was expressed in area under the curve (AUC; calculated with the trapezoidal rule) and incremental AUC (ΔAUC; area above baseline during the interval) for 60 min after glucose injection (glucose-stimulated insulin, $\mu U \cdot ml^{-1} \cdot 60$ min), and the acute (first-phase) insulin response (AIR; $\mu U \cdot ml^{-1} \cdot 3$ min) was expressed as ΔAUC for 3 min after glucose injection. Postprandial studies were expressed in AUC and ΔAUC during 5 h postprandial glucose (mM . 5 h), insulin ($\mu U \cdot ml^{-1} \cdot 5$ h), and glucagon ($pg \cdot ml^{-1} \cdot 5$ h), and in absolute levels or incremental values of CCK and PP (pM) at 60 min. Results of intravenous bombesin studies were

expressed in the level and incremental value of PP at 5 min after injection.

Analytical procedures

Plasma glucose was measured by the glucose oxidase method. Blood samples for the determination of hormone levels were collected on ice with aprotinin (1000 KIU/ml blood, Trasylol, Bayer, Leverkusen, West Germany), centrifuged at 4°C, and stored within 15 min at -20°C (or -70°C for glucagon), pending assay. The detailed methods used for radioimmunoassay of insulin [11], PP [12], and CCK [13] have been reported previously. Plasma glucagon was radioimmunoassayed with a specific antiserum to a synthetic pancreatic glucagon that does not cross-react with enteroglucagon. Antibody-bound radioactivity was separated from free label by the double-antibody technique via goat anti-rabbit γ-globulin. Reagents and method were obtained from Daiichi (Tokyo). Sensitivity was 15 pg/ml and intra- and interassay variation were 6.4 and 7.1%, respectively.

Statistical analysis

Results are expressed as means ± SE. Logarithmic transformation of data was used when appropriate to normalize the distribution of the data. All response curves and comparison of means at different intervals (for group 2), were evaluated by single-factor analysis of variance with repeated measures. Multiple comparisons were performed with Scheffé's test and Fisher's protected least-significant-difference test. Unless stated otherwise, Student's paired and unpaired two-tailed t tests were used as applicable. Differences were considered NS at $p > .05$.

Results

All animals were in good clinical condition throughout the observation period and did not lose a significant amount of weight. The results of fasting, postprandial, IVGTT, and bombesin-infusion studies are summarized in Table V.1. Response curves in controls and 6 wk after partial pancreatectomy in experimental dogs are compared in Fig. V.1. The 6-wk curves after partial pancreatectomy are shown again in Fig. V.2 for comparison with response curves after venous transposition and duct obliteration in these dogs.

Table V.1

Effects of partial pancreatectomy, diversion of venous drainage to systemic circulation (shunt), and duct obliteration of these systemically draining remnants on glucose regulation in dogs

Parameters	Group 1 (n = 14)	Group 2 (n = 6)		
		70% Pancreatectomy	Shunt	Duct obliteration
Fasting*				
Glucose (mM)	5.9 ± 0.1	5.8 ± 0.1†	5.6 ± 0.1†	6.0 ± 0.2
Insulin (μU/ml)	13 ± 1	12 ± 2†	23 ± 2	17 ± 2
Glucagon (pg/ml)	77 ± 18	61 ± 10	66 ± 15	64 ± 14
CCK (pM)	2.3 ± 0.3	2.7 ± 0.4†	1.2 ± 0.2	1.8 ± 0.6
PP (pM)	57 ± 4	52 ± 6†	38 ± 5	30 ± 4
IVGTT				
K_G value (–%/min)	3.3 ± 0.2†	2.3 ± 0.6	1.9 ± 0.2†	1.0 ± 0.1
Glucose-stimulated insulin (μU/ml)‡	2775 ± 282†	1766 ± 294†	4291 ± 674†	2165 ± 235
ΔAUC	2095 ± 205†	983 ± 273†	2779 ± 602†	1075 ± 151
AIR	184 ± 18	149 ± 59	184 ± 23†	66 ± 11
Postprandial				
Glucose (mM)§	30 ± 1	31 ± 1	31 ± 1†	40 ± 4
ΔAUC	0.9 ± 0.2	1.5 ± 0.3	3.0 ± 0.8	10.4 ± 3.8
Insulin (μU/ml)§	244 ± 22	198 ± 37†	395 ± 46	312 ± 35
ΔAUC	170 ± 23	146 ± 33†	304 ± 62	231 ± 34
Glucagon (pg/ml)§	593 ± 87	440 ± 44	557 ± 78	613 ± 87
ΔAUC	237 ± 30	167 ± 19	232 ± 65	301 ± 47
CCK (pM)‖	9.1 ± 1.6	7.2 ± 1.2	5.6 ± 0.9	5.5 ± 0.6
Δ60 min	6.3 ± 1.6	4.9 ± 1.4	4.8 ± 0.7	3.8 ± 0.6
PP (pM)‖	364 ± 30	280 ± 49	222 ± 16†	70 ± 17
Δ60 min	303 ± 30†	199 ± 24	178 ± 10†	40 ± 18
Bombesin-stimulated				
PP (pM)¶	213 ± 34	106 ± 24	81 ± 19†	29 ± 4
Δ5 min	160 ± 32†	50 ± 16	45 ± 16†	2 ± 2

Group 1 served as healthy unmodified controls. Data are means ± SE. Details of statistical evaluations are given in RESULTS. CCK, cholecystokinin; PP, pancreatic polypeptide; IVGTT, intravenous glucose tolerance test; ΔAUC, incremental area under the curve; AIR, acute insulin response; Δ60 min, Δ5 min, increment over basal. *Individual fasting levels were calculated as means of values obtained on 2 consecutive days. † $p \leq .05$ compared with values in the same row and next column to the right. ‡ Expressed in AUC of plasma insulin for 60 min after intravenous glucose bolus infusion. AIR is expressed in ΔAUC for 3 min after glucose bolus injection. § Expressed in AUC of response during 5 postprandial h. ‖ Expressed in plasma levels at 60 min. ¶ Expressed in plasma levels at 5 min

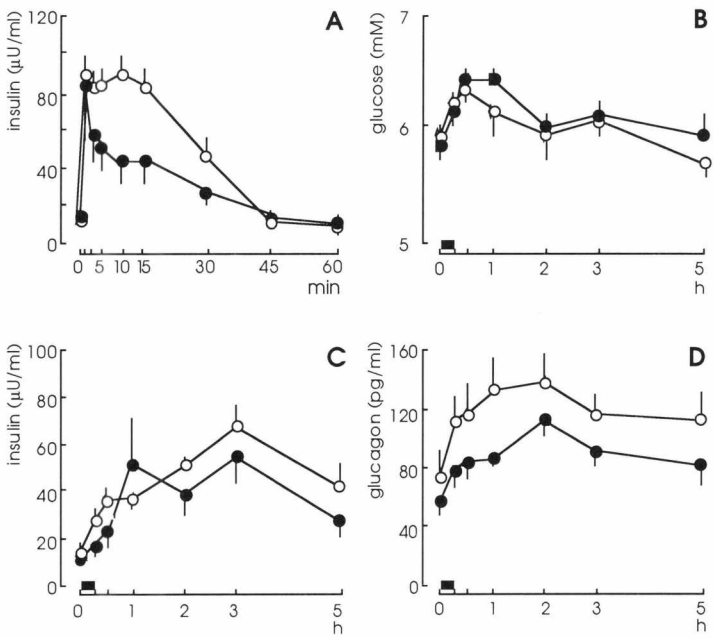

Fig.V.1

*The plasma insulin response to i.v. bolus glucose (0.5 g/kg) injection (**A**), and postprandial curves of plasma glucose (**B**), insulin (**C**) and glucagon (**D**) in normal unmodified dogs (open circles; n =14) and ~70% pancreatectomized dogs (closed circles; n =6). Data are expressed as means ± SE.*

Partial pancreatectomy

Fasting glucose and hormone levels were unaffected by partial pancreatectomy (Table V.1). K_g values modestly declined (30%), and a concomitant reduction was observed with the insulin response at IVGTT. Glucose-stimulated insulin values (AUC) 6 wk after partial pancreatectomy were ~33% lower than those observed in control animals (p <.02). Incremental insulin values (ΔAUC) were more markedly diminished (p <.001), but the AIR was not significantly affected (Table V.1; Fig. V.1A). Thus, the second-phase insulin response was particularly reduced. Postprandial glucose, insulin, and glucagon levels were not affected by partial pancreatectomy (Table V.1; Fig. V.1B–D). Both before and after partial pancreatectomy, postprandial CCK (p <.05), PP (p <.001), and intravenous bombesin-stimulated PP (p <.02) levels had significantly increased

over corresponding basal levels. However, partial pancreatectomy significantly reduced the incremental postprandial and bombesin-stimulated PP values (p <.05).

Diversion to systemic circulation

After venous transposition with diversion of the pancreatic remnant venous effluent from portal to systemic circulation, fasting levels of glucose , CCK, and PP declined (p <.05), fasting glucagon did not change appreciably, and fasting insulin rose twofold (p <.01). K_g values had not significantly changed. Except for the AIR, a significant two- to threefold increase of glucose-stimulated insulin values, both for AUC (p <.01) and ΔAUC (p <.02), was observed after venous transposition (Table V.1; Fig. V.2A). Postprandially, glucose and glucagon values had not changed appreciably after venous transposition (Table V.1; Fig. V.2, B and D) and again, as in fasting and IVGTT studies, both AUC (p <.02) and ΔAUC (p <.05) insulin values had increased markedly after venous transposition (Table V.1; Fig. V.2C). Postprandial CCK, PP, and intravenous bombesin-stimulated PP levels, like corresponding basal levels, showed a tendency to decrease (NS, Table V.1). However, a significant increase over basal levels could be demonstrated (p <.02), and corresponding incremental values remained essentially unchanged.

Duct obliteration

Duct obliteration of the systemically draining pancreatic remnants clearly affected both glucose metabolism and endocrine performance. An increase in fasting blood glucose levels was observed (p <.05) with a tendency for the fasting insulin levels to decline (NS, Table V.1). The basal release of other hormones was not significantly affected. A conspicuous reduction of both K_g values (p <.01) and the insulin response (p <.02) at IVGTT was observed (Table V.1). The acute, first-phase, insulin response amounted to only ~ 30% of values obtained before obliteration (p <.005), and insulin response curves demonstrated compensatory second-phase release (Fig. V.2A). A highly significant sustained increase (p <.001) over basal insulin up to 60 min after glucose injection could be demonstrated, whereas before obliteration (at intervals 1 and 2) basal values had been attained within 30 min. Postprandial glucose increased (p <.05), and corresponding incremental values nearly tripled, although no statistical confirmation was obtained (Table V.1). Glucose profiles after a test meal demonstrated a rise over basal glucose from 15 min (p <.05) with sustained hyperglycemia (mean ~ 8 mM) during the test (p <.01; Fig.

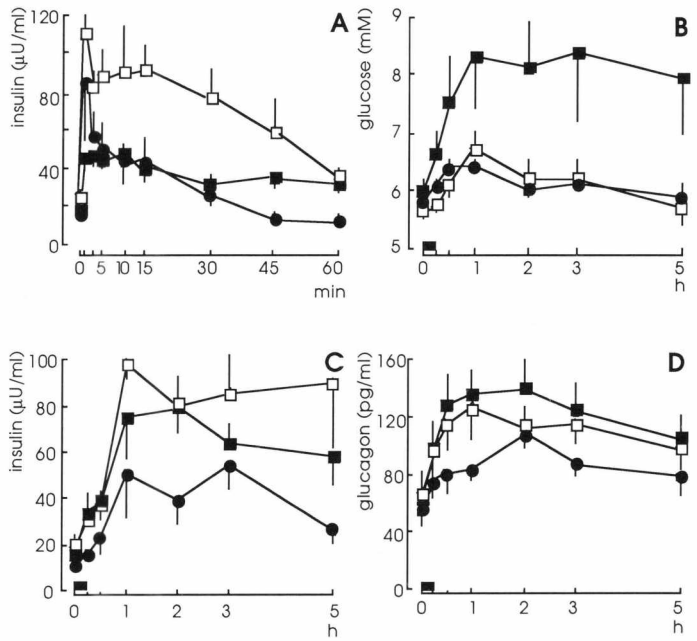

Fig.V.2

*The plasma insulin response to i.v. bolus glucose (0.5 g/kg) injection (**A**), and postprandial curves of plasma glucose (**B**), insulin (**C**) and glucagon (**D**) in experimental dogs (n =6) at three successive intervals: 6 wk after ~70% pancreatectomy (closed circles), 2 wk after diversion of venous drainage from portal to systemic circulation (open squares), and 6 wk after duct obliteration (closed squares) of systemically draining pancreatic remnants. Data are means ± SE. Data from ~70% pancreatectomized dogs studied at 6 wk are same as presented in Fig. V.1.*

V.2B). In contrast, postprandial insulin, glucagon, and CCK were quantitatively unaffected (Table V.1; Fig. V.2, C and D). Postprandial PP values were markedly reduced ($p < .02$), however, a significant rise over basal levels could still be demonstrated ($p < .05$). In contrast, bombesin failed to stimulate PP secretion after duct obliteration.

Discussion

In a recent study we showed that the insulin-secreting capacity of the splenic segment of the canine pancreas is reduced to 25% of its initial value after duct-

obliterated segmental autotransplantation if an intravenous glucose bolus injection was administered under general anesthesia [11]. That study did not address the individual contribution of partial pancreatectomy, venous transposition, and duct obliteration to this decrease in endocrine function. Other groups either studied the separate effects of these modifications in separate groups of animals or a combination of these measures compared with healthy unmodified controls. It has been emphasized that both duct-occlusion-induced fibrosis [10,11,14-17] and pancreatic mass reduction [18-21] are major determinants in the deterioration of glucose regulation in previous experiments. In this study we addressed the separate and additive effects of these aspects of segmental pancreas transplantation in a crossover design.

Carry-over effects are the obvious potential disadvantages of a crossover design. To minimize effects of potential progressive adaptation to previous treatments, glucose regulation was tested as soon as possible, 2 wk after the animals had recuperated from venous transposition and again at 6 wk after duct obliteration, because duct obliteration has been found to be associated with stable, albeit reduced, endocrine pancreatic function from 1 mo postoperation [10].

The main advantage of the model used in this study is that the regular enteric exocrine drainage from the pancreatic remnant could be left intact, in contrast to previous canine studies that used the tail or splenic segment of the pancreas. Studies of the duct-obliterated splenic segment in the conscious animal do not have adequate controls, i.e., the nonobliterated segment, because with pancreatic segments draining freely into the peritoneal cavity, fibrosis, after the spontaneous gradual occlusion of the duct, may interfere with islet morphology [22] and function [15,16,18-21].

After partial pancreatectomy, we observed a reduction in intravenous glucose tolerance and the second-phase insulin response at IVGTT. Our data are supported by findings in similar dog models of 50-70% partial pancreatectomy [23,24]. These groups also studied the duodenal remnant and observed no significant changes in the acute intravenous glucose-stimulated insulin response, a reduced intravenous glucose tolerance [23] and a reduced second-phase insulin response to intravenous glucose [24]. In contrast a reduced or abolished acute insulin response has been a general finding in dog studies of the intraperitoneal free-draining splenic segment after resection of the duodenal pancreas [21,25,26].

These conflicting results may be explained by changes of innervation to the remnant pancreas. The canine pancreas is innervated along different routes: the major supply of vagal parasympathetic fibers mixed with sympathetic fibers

originates from a plexus in the region of the duodenal bulb and enters the parenchyma of the duodenal pancreas. The majority of sympathetic fibers originate from the celiac and superior mesenteric ganglia and enter the parenchyma of the splenic segment. There are probably some parasympathetic nerves running along this course as well [27]. Thus, in this study, intact parasympathetic and at least partially intact sympathetic innervation of the remnant were present after partial pancreatectomy, whereas previous work studying the canine splenic lobe added virtually total parasympathetic denervation to mass reduction by resection of the duodenal pancreas.

Because direct vagal potentiation of intravenous glucose-stimulated insulin release has been demonstrated to be exclusively associated with the acute insulin response [28], an intact acute insulin response in this and other studies of the duodenal remnant as opposed to a dulled insulin response in studies of the splenic pancreatic segment may be explained from the intact vagal innervation of the duodenal remnant. Likewise, because meal- and bombesin-stimulated PP is known to be largely dependent on vagal cholinergic mechanisms [5,6,9], the significant PP response to these stimuli in our study, as opposed to the complete abolition of the PP response to these stimuli in studies of the innervated [29] and denervated [16,30] splenic pancreatic segment after resection of the duodenal segment, may be explained from transection of afferent cholinergic nerve fibers to the splenic pancreatic remnant.

The modest reduction in stimulated PP values after partial pancreatectomy here is likely to be the result of a reduction in PP cell mass. Although partial sympathetic denervation of the pancreatic remnant in our model cannot be excluded, rather an increase of intravenous glucose-stimulated insulin would have been expected after sympathectomy [25,31,32]. Thus, reduction of the second-phase insulin response also has yet to be ascribed to pancreatic mass reduction as such. Remarkable retrospective clinical data were gathered recently by Kendall et al. [33], who demonstrated that even the preoperative second-phase insulin response with IVGTT is a sensitive parameter predicting future deterioration of glucose regulation in living related donors of splenic pancreatic segments.

In pancreas transplantation, venous drainage to the systemic circulation is preferred for technical reasons. Both improvement of intravenous glucose tolerance after systemic delivery of pancreatic hormones [10, 34,35,37] and no effects [26,36-38] have been reported in several other studies with solid organs, islet grafts, or glucose-controlled insulin infusion systems (GCIIS). Although in this study, peripheral glucose-stimulated insulin levels increased significantly after venous transposition, no changes in glucose tolerance were observed. In a

previous study, we presented data suggesting that higher peripheral insulin levels after venous transposition would lead to more rapid disappearance of glucose from the peripheral circulation and thus to an increase in $K_g$values [10]. Differences in experimental protocol may explain these inconsistent results. In this study, the short-term effects of venous transposition — and the addition of duct obliteration to venous transposition — were studied with the nonobliterated segmental pancreas; whereas previously, portal and systemic drainage from the long-term obliterated segmental pancreas were compared. The doubled to tripled peripheral insulin values after venous transposition with systemic delivery of pancreatic hormones is undoubtedly at least partially the result of bypassing of first-pass hepatic insulin extraction, because the liver has been shown to be the major site of insulin breakdown [39-42]. Similar observations have been made with diversion from portal to systemic circulation, or vice versa, of the venous effluent of canine islet grafts [36,43] and in a recent study of the canine intraperitoneal free-draining splenic pancreatic segment by Krusch et al. [26]. The failure of glucagon and PP levels to rise after venous transposition confirms previous reports on the hepatic contribution to removal of these pancreatic hormones [39-41, 44].

Of special interest is the observed decline of basal CCK and PP levels after venous transposition. It is possibly that the decline of basal CCK levels may be attributed to the concomitant increase of peripheral insulin levels. Similarly, it has been suggested that insulin exerts a negative feedback on the basal and stimulated glucose-dependent insulinotropic polypeptide, another gut hormone involved in the enteroinsular axis [45,46]. However, with a reduction of fasting glucose rather an increase of PP secretion would be expected. The observed decrease of basal PP levels on venous transposition might be explained from the reduced CCK levels, because intravenous CCK is known to stimulate PP secretion [47].

The most dramatic changes in glucose regulation were observed after in situ duct obliteration. Duct obliteration induced sustained, fasting, and postprandial hyperglycemia. At IVGTT, a 50% reduced K_g value was associated with a 50% decrease in the peripheral insulin response and a 70% decrease in the AIR. These findings in the conscious animal corroborate our previous findings with IVGTT in the anesthetized animal demonstrating a 75% reduction of intraoperative insulin output in the pancreatic venous effluent at 6 wk after duct-obliterated segmental autotransplantation [11].

Although there are convincing data to show that duct obliteration interferes with endocrine function, it has not yet been clarified how glucose regulation is affected. A mean 50% reduction of intravenous glucose-stimulated insulin

contrasts with postprandial normoinsulinemia compared to the unmodified caval draining segment or hyperinsulinemia compared to the portal draining segment. Postprandial normoinsulinemia with duct-obliterated remnants rather than a severely reduced intravenous glucose-stimulated insulin response may be explained both from the different stimuli with these tests, as well as from the postprandial contribution of the enteroinsular axis. Although the role of CCK as an incretin is controversial with respect to the human species [45,48], evidence has been presented that CCK acts synergistically with GIP [49] and by itself may potentiate insulin secretion in other species including the dog [50,51]. Since the postprandial CCK response was not affected by duct obliteration with exocrine substitution and since CCK is known to have a progressively greater stimulatory effect on insulin release with higher glucose levels [52,53], normo- to hyperinsulinemia might be attained only as a consequence of a postprandial hyperglycemia enhanced incretin effect. Since postprandial insulin in the present study was not significantly affected, it is concluded that glucose-sensitivity rather than the insulin secreting capacity is involved.

Although postprandial hyperglycemia as outlined above may explain postprandial normoinsulinemia in contrast to reduced intravenous glucose-stimulated insulin after duct obliteration, these glucose excursions remain to be explained. Because quantitatively peripheral postprandial insulin was not affected by duct obliteration, although insulin delivery was insufficient with respect to the prevailing glycemia, and because intravenous glucose-stimulated acute release was affected, fine regulation of insulin release seems deranged. A growing body of evidence suggests that an intrinsic autonomously functioning intrapancreatic neuronal network coordinates the secretory activity of islets to produce pulsatile peptide secretion [for review, see ref. 54]. Intact islet architecture is probably also required for these short term oscillations [55]. Previous studies demonstrated that duct obliteration, apart from inducing atrophy of exocrine tissue, disrupts islet architecture [14,22]. Our finding that the PP response to bombesin stimulation was abolished by duct obliteration suggests intrinsic denervation of islets as an important effect of duct obliteration. This suggestion is supported by a remarkable fall of the first-phase insulin response. Meal-stimulated PP secretion was only partly inhibited by duct obliteration because postprandially released PP is mediated by both vagal cholinergic nerve fibers and stimulatory enteral hormones, especially CCK [6,47]. To date, no postprandial increments in bombesinlike peptides have been observed in plasma and because these peptides are primarily located in gastroenteric plexuses and nerve fibers innervating intrapancreatic ganglia and islets [7,8], bombesin-like peptides may have a neurotransmitter role in

physiology. Both a cholinergic-mediated response and direct bombesinergic action at the level of pancreatic intrinsic plexuses or PP cells may participate in intravenous bombesin-stimulated PP [5,6,9]. Thus, because basal PP levels were not affected and a significant postprandial increment was still observed, a substantial mass of viable PP cells was still present and it therefore follows that intrinsic denervation must be implicated in the abolished response to intravenous bombesin.

Because duct obliteration mimics the effects of denervation on ß-cell and PP cell function and interferes with normal islet morphology, we suspect that obliteration-induced intrinsic denervation of ß-cells interferes with normal pulsatile insulin delivery, which would explain the postprandial hyperglycemia with normo- or hyperinsulinemia. Pulsatile compared with nonpulsatile insulin delivery with GCIIS [54] requires far less insulin to obtain normoglycemia. Nonpulsatile insulin secretion has indeed been observed with pancreatitis and acinar fibrosis in humans [54,56,57]. Although pancreas transplantation necessitates extrinsic denervation, intrinsic innervation [58-61] and intrinsic fine regulation of insulin release might survive in the nonobliterated graft. Whether such a mechanism exists requires further investigation.

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