Kinetics of Intraperitoneally Infused Insulin In Rats

Functional Implications for the Bioartificial Pancreas

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Intraperitoneal transplantation of encapsulated islets can restore normoglycemia in diabetic recipients but not normal glucose tolerance nor normal insulin responses to a physiological stimulus. This study investigates whether the intraperitoneal implantation site as such contributes to the interference with optimal transport kinetics between the islets and the bloodstream. Insulin was infused into the peritoneal cavity of conscious and freely moving rats in doses of 20, 40, and 80 pmol · l^{-1} · min^{-1} during 15 min, to mimic the gradual release of insulin from an encapsulated, i.e., a nonvascularized, islet graft. With 20 pmol · l^{-1} · min^{-1}, we observed virtually no rise of insulin levels, and it took 30 min until glucose levels had dropped significantly. With 40 and 80 pmol · l^{-1} · min^{-1} insulin infusions, there was a dose-dependent rise of insulin and decrease of glucose levels. When compared with intraportal infusions with the same insulin dosages, however, they were strongly delayed and reduced as well as prolonged. Similar results were obtained when inulin instead of insulin was intraperitoneally infused, which indicates that the transport of insulin from the peritoneal cavity to the bloodstream is mainly by passive diffusion. With a view on the clinical efficacy of the bioartificial pancreas, our findings indicate that we should focus on finding or creating a transplantation site that, more than the unmodified peritoneal cavity, permits close contact between the bloodstream and the encapsulated islet tissue. *Diabetes* 45:1102–1107, 1996

Transplantation of microencapsulated pancreatic islets is a promising concept for the cure of diabetes, since it may allow for deleting immunosuppressive therapy and for using grafts from xenogeneic sources which are always sufficiently available. As a consequence of its volume, a microencapsulated islet graft can only be implanted into the peritoneal cavity. Normoglycemia has been reported after intraperitoneal transplantation of microencapsulated islets both in chemically induced and in autoimmune diabetic animal models (1–5) and recently also in humans (6). But a previous report from our laboratory showed that glucose tolerance remains disturbed in spite of normoglycemia and that there is no increase of plasma insulin levels in response to a physiological stimulus such as the intake of a meal (5). This may be due to several factors such as a gradual decrease of the number of viable and functioning islets after transplantation as a consequence of fibrotic overgrowth of the capsules, or a slow release of insulin in response to a glycemic stimulus as a consequence of too large capsule diameters (7).

The transplantation site as such may be another factor. Previous studies report the delay between intraperitoneal administration of insulin and rise of plasma insulin levels to vary between only 1 to 10 min (8–11). This would imply that there is no significant barrier (12–14) for the exchange of insulin between peritoneal fluid and blood and, consequently, no significant contribution of the transplantation site as such to the absence of increased plasma insulin levels after glucose challenge in recipients of intraperitoneally located microencapsulated islet grafts. Notably, however, these previous studies on the kinetics of intraperitoneal insulin absorption (8–11) were often performed with insulin bolus injections in unphysiologically high doses, which may well be associated with a more rapid absorption of insulin than should be expected with a gradual release of insulin from encapsulated pancreatic islets. Therefore, the present study was undertaken to determine the rate of uptake of insulin in the blood after gradual infusion into the peritoneal cavity of varying quantities of insulin. These profiles were compared with plasma insulin profiles after insulin infusion into the portal vein, which mimic the physiological situation. Furthermore, plasma insulin profiles after intraperitoneal insulin infusion were compared with plasma inulin profiles after intraperitoneal inulin infusion to decide whether the mode of transfer of insulin from the peritoneal cavity to the bloodstream is by simple diffusion.

RESEARCH DESIGN AND METHODS

Animals and surgery. Male inbred Albino Oxford (AO/G) rats weighing 340–360 g were obtained from the Central Animal Laboratory of Groningen and kept under standard laboratory conditions. Rats were provided with four permanent silicon catheters for blood sampling and infusion in unanesthetized, undisturbed, and freely moving animals (15). The catheters were inserted in two separate surgical procedures under halothane anesthesia. During the first procedure, catheters were placed into the right jugular vein and the left jugular vein (16) and into the portal vein (17) for blood sampling, for infusion, and for both blood sampling and infusion, respectively. A fourth catheter was temporarily kept under the skin of the abdominal wall. The intraportal insulin infusions and the intravenous inulin infusions were performed after the animals had reached their preoperative weight.

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AUCins(tot), area under the curve from the first measurement above basal insulin values until basal values were reached again; PC, portal circulation; SC, systemic circulation.

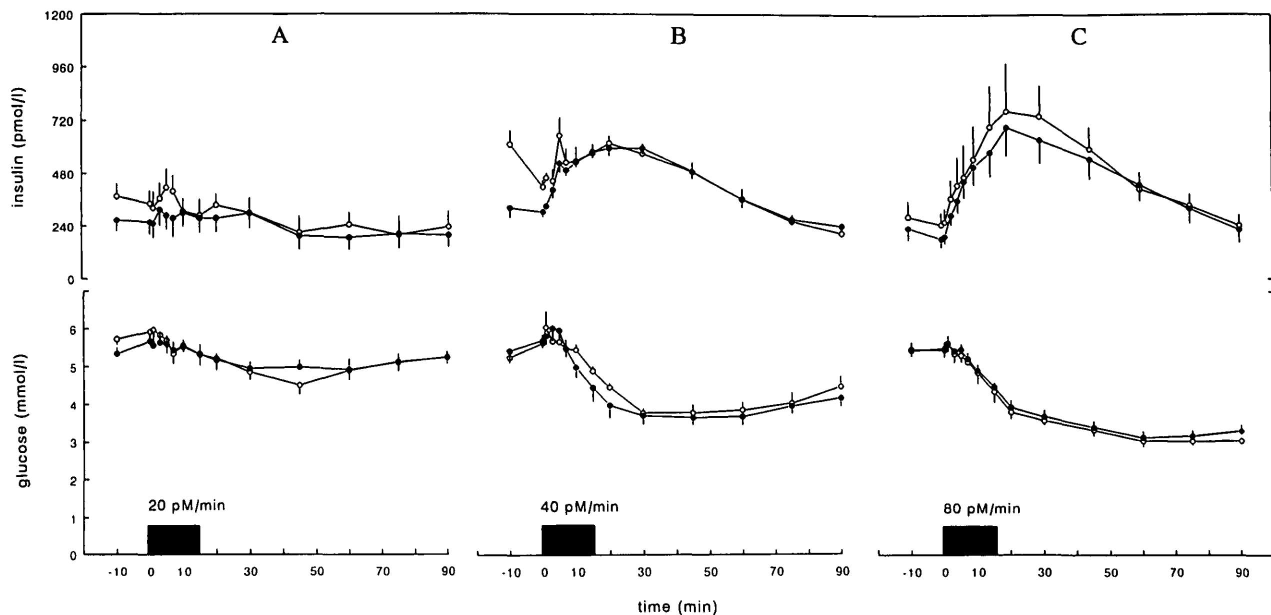

FIG. 1. Effect of intraperitoneal infusion of different concentrations of insulin on the portal (○) and systemic (●) plasma insulin and blood glucose levels. Insulin was infused in a dose of 20 pmol · l^{-1} · min^{-1} (*A*, *n* = 5), 40 pmol · l^{-1} · min^{-1} (*B*, *n* = 4), and 80 pmol · l^{-1} · min^{-1} (*C*, *n* = 6) during 15 min. Values are means ± SE. With 20 pmol · l^{-1} · min^{-1} blood glucose levels decreased significantly as of 30 min after the start of the infusion ($P < 0.02$) and remained decreased in both PC and SC ($0.009 < P < 0.05$) until t = 75 min. Blood glucose levels with 40 and 80 pmol · l^{-1} · min^{-1} decreased both in the PC and the SC as of 15 min after the start of the infusion and continued to be low throughout the experiment ($0.004 < P < 0.03$). The insulin infusion of 40 and 80 pmol · l^{-1} · min^{-1} induced a significant rise of insulin levels in both the PC and the SC within 7 ($P < 0.03$) and 5 ($P < 0.03$) min, which returned to basal levels levels after 60 min ($0.02 < P < 0.03$) and 75 min ($0.02 < P < 0.04$), respectively.

During a subsequent second procedure, the subcutaneous catheter was provided with a Teflon tip and brought into the cranial left quadrant of the abdominal cavity near the umbilicus, which reportedly is an efficient site for insulin absorption (18). The intraperitoneal infusions were performed 2 days later. Free flow through the catheter was verified in all animals by infusing India ink through the intraperitoneal catheter during a third laparotomy under anesthesia. The ink was always and readily observed to spread over the omentum, the visceral organs, the parietal peritoneum, and the lower liver lobuli.

Infusions and determinations. Insulin (Actrapid, human insulin, Novo Nordisk, Bagsværd, Denmark) was diluted in sterile saline containing 3% bovine serum albumin. Insulin was administered during 15 min by either intraperitoneal or intraportal infusion of 300, 600, and 1,200 pmol/l of insulin at a rate of 20, 40, and 80 pmol · l^{-1} · min^{-1}, respectively. Inulin (extracted from dahlia tubers, M_r 5,000, Sigma, St Louis, MO) was infused at a rate of 2 mg/min during 15 min.

The animals were fasted for 2 h before and during the experiments. Blood samples were taken at 10 min and immediately before insulin administration to determine basal values, and subsequent samples were taken at 1, 3, 5, 7, 10, 15, 20, 30, 45, 60, 75, and 90 min after the start of insulin infusion. Blood lost during sampling was replaced by transfusing blood from a donor rat at regular intervals.

Blood glucose concentrations were determined in whole blood by a ferricyanide method with a Technicon autoanalyzer. Plasma insulin was measured by radioimmunoassay, with rat insulin as a standard. Plasma inulin was measured according to Davison and Sackner (19).

The amount of insulin responsible for the total plasma insulin elevation was quantified by calculating the area under the curve from the first measurement above basal value until basal values were reached again [AUCins(tot)].

Statistical analysis. Results are expressed as means ± SE. Statistical comparisons between plasma and insulin levels were made with the Mann-Whitney *U* test. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of intraperitoneal insulin infusion. Intraperitoneal infusions per se had no effect on plasma insulin levels nor blood glucose levels (data not shown) as verified by infusing 1.5 ml saline in 15 min. During the whole experiment, both basal insulin and glucose levels were somewhat higher in the portal circulation (PC) than in the systemic circulation (SC).

After intraperitoneal insulin infusion of 20 pmol · l^{-1} · min^{-1} (Fig. 1*A*), the difference of insulin concentration between the PC and the SC disappeared within 10 min, but there was no rise in plasma insulin. Blood glucose levels were observed to decrease significantly in both PC and SC as of 30 min after the start of the infusion.

The insulin infusion of 40 pmol · l^{-1} · min^{-1} (Fig. 1*B*) and 80 pmol · l^{-1} · min^{-1} (Fig. 1*C*) induced a significant rise of insulin levels in both the PC and the SC within 7 and 5 min, respectively. Blood glucose levels decreased both in the PC and the SC from 15 min after the start of the infusion for both doses of insulin. They continued to be low throughout the experiment with only slow and gradual increases from 60 min after the start of the experiment.

Comparison of intraperitoneal and intraportal insulin infusion. The plasma insulin and blood glucose levels in the SC obtained with intraperitoneal infusion of insulin as described in the preceding section were compared with plasma insulin and blood glucose levels in the SC obtained with intraportal infusion of insulin. To this end, both the plasma insulin and the blood glucose levels were expressed as Δ insulin and Δ glucose, respectively, with the t = 0 levels taken as the zero reference.

As is clear from Fig. 2, the plasma insulin responses to intraperitoneal infusion of insulin lagged far behind the responses to intraportal insulin infusion and were much lower. Glucose concentrations in the SC responded slower to intraperitoneal than to intraportal insulin infusions. The responses to the 20 pmol · l^{-1} · min^{-1} intraperitoneal insulin infusions were not only slower but also less profound than the responses to intraportal infusions (Fig. 2*A* and *B*). But with the 40 and 80 pmol · l^{-1} · min^{-1} intraperitoneal insulin infusion, the blood glucose was, although with a delay of 30 min, reduced to levels similarly low as those observed with

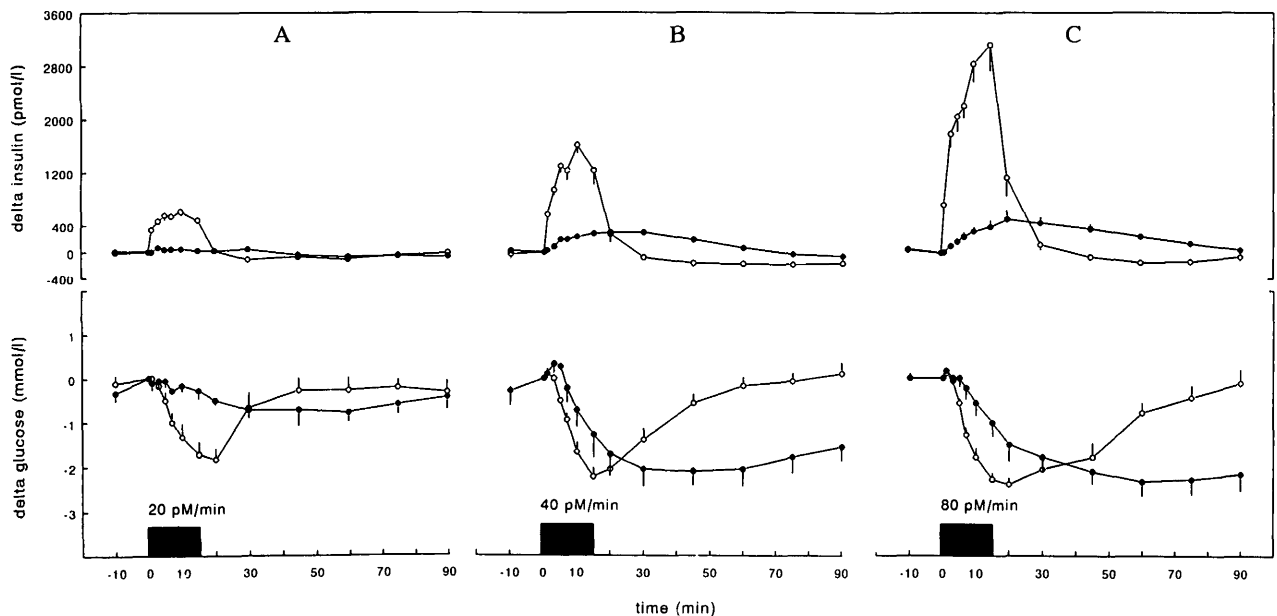

FIG. 2. Effect of intraperitoneal (●) and intraportal (○) infusion of different concentrations of insulin on plasma insulin and blood glucose levels. Insulin was infused intraportally in a dose of 20 pmol · l^{-1} · min^{-1} (*A*, $n = 6$), 40 pmol · l^{-1} · min^{-1} (*B*, $n = 5$), and 80 pmol · l^{-1} · min^{-1} (*C*, $n = 7$) during 15 min. Values are means ± SE of levels expressed as Δ levels with the $t = 0$ level taken as the zero reference. For intraperitoneal infusion, the data presented here are the means of the Δ levels of the systemic plasma insulin and blood glucose levels as presented in Fig. 1.

the 40 and 80 pmol · l^{-1} · min^{-1} intraportal insulin infusions (Fig. 2*C*).

As shown in Fig. 3, the total amount of insulin delivered into the SC after 40 and 80 pmol · l^{-1} · min^{-1} infusion was approximately twofold lower with the intraperitoneal than with the intraportal insulin infusions. The AUCins(tot) after intraperitoneal infusion of 20 pmol · l^{-1} · min^{-1} insulin was virtually absent.

Comparison of insulin and inulin infusion. The patterns of appearance of insulin in the SC obtained with intraperitoneal and intraportal infusions of insulin were compared with those of inulin obtained with intraperitoneal and intravenous infusions of inulin. Infusion of inulin had no effect on the glucose concentrations (data not shown). As graphically illustrated in Fig. 4, intraperitoneal infusion of inulin induced a slower and more continuous appearance of plasma inulin than intravenous infusion. The pattern of appearance in plasma of intraperitoneally infused inulin was similar to that of intraperitoneally infused insulin, a similarity most conspicuously illustrated by comparing Fig. 4 with Fig. 2*C*.

DISCUSSION

Our experiments to study the rate of uptake of insulin after gradual infusion into the peritoneal cavity were performed in nondiabetic animals rather than in diabetic animals to avoid enhanced absorption of insulin by the tissues (20,21) and diminished uptake of intraperitoneal insulin in the blood (20–23). It is unlikely that endogenous insulin has contributed to the patterns of uptake of the infused insulin since insulin administration is associated with a decrease of the blood glucose levels, which suppresses the endogenous production of insulin (24,25). Parameters known to influence the rate of uptake of insulin were chosen to mimic the situation in recipients of an encapsulated islet graft. Thus, rats with similar body weights were used as recipients of successful microencapsulated islet grafts (5). Also, insulin was delivered in a 1.5-ml volume, which is similar to that of a microencapsulated islet graft (5,7). This implies that the volume of delivery in this study was similar to the volume over which the insulin released by an encapsulated islet graft is distributed. Should insulin be infused in a smaller volume and, consequently, in a higher concentration, a faster uptake of the insulin would be expected (9).

There are two explanations for the strong delay of insulin appearance in plasma after intraperitoneal infusion. First, plasma appearance of intraperitoneal insulin is delayed by nonspecific and reversible binding of insulin to the mesentery or other intra-abdominal organs (14,22,23,26). Another and probably more significant explanation is that passive diffusion plays a major role in transport, as previously suggested by Rubin et al. (27), Balducci et al. (28), and Wideröe et al. (29). Now this is confirmed by our observation that the plasma appearance curves of insulin are similar and almost identical to those of inulin. This is a carbohydrate molecule of similar size and molecular weight as insulin, which freely distributes between the intravascular and ex-

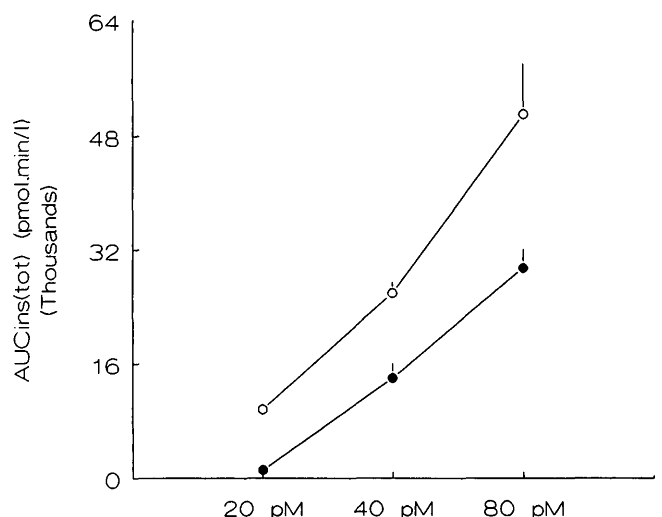

FIG. 3. AUCins(tot) after intraperitoneal (●) and intraportal (○) insulin infusion. Values are means ± SE.

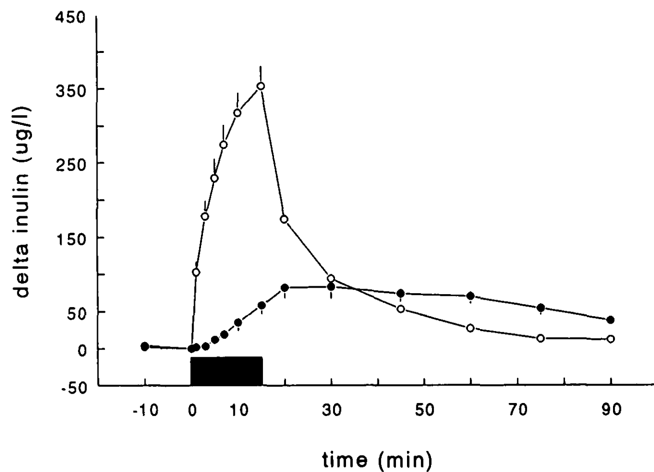

FIG. 4. Effect of intraperitoneal (●) and intravenous (○) infusion of inulin on plasma inulin levels in the SC. Inulin was infused at a concentration of 2 mg/ml during 15 min. Values are means ± SE.

travascular compartments by diffusion through capillary pores (30–32).

The passive diffusion depends upon the building of a concentration gradient between the peritoneal cavity and the peritoneal microcirculation. This gradient is built rather slowly when insulin is administered by prolonged infusions during 15 min as in our experiments, which were designed to mimic the rather gradual release of insulin from an intraperitoneal bioartificial pancreas, i.e., a nonvascularized islet graft. Consequently, intraperitoneal infusion of insulin induces only a moderate increase of plasma insulin levels, which confirms findings of Poulsen et al. (33) and Selam et al. (34). However, our observations do not corroborate the explanation of the latter authors that a large portion of the infused insulin is removed during its first pass through the liver, since we observed equal portal and systemic insulin concentrations after intraperitoneal infusion (Fig. 2). Neither do our findings appear to corroborate those of Schade et al. (9), Micossi et al. (35), and Radziuk et al. (36), who observed intraperitoneally infused insulin to appear in the blood rapidly (within 1 min) and in high concentration. These authors, however, did not administer the insulin by infusion but by bolus injection, which is associated with a pronounced concentration gradient and, consequently, with a rapid flux of insulin into the circulation (37,38). The slow absorption patterns of gradually infused insulin corroborate the clinical data on the kinetics of insulin absorption with pump administration (18,38) and on the absorption of insulin from dialysis fluids in diabetics on ambulatory peritoneal dialysis (27–29).

Our findings seem to contradict those of others (9,36,39) who observed a positive portal-systemic insulin gradient with intraperitoneal insulin administration, while we found the spontaneous gradient to disappear within the first 10 min of intraperitoneal insulin administration and to remain absent during the subsequent 80 min of repeated sampling. This should largely be explained by the fact that we did not administer the insulin by bolus injection but by slow infusion during a prolonged period of time. As a consequence, local intraperitoneal insulin concentrations did not reach high peak levels as with bolus injection. Slow infusion, therefore, is associated with slow absorption into the portal system. The absorbed insulin does not induce a significant rise in the portal-systemic insulin gradient but is rather restricted to temporarily maintaining the spontaneous gradient. This gradient disappears with continuing infusion, not only because systemic insulin levels increase but also because these increased levels induce hypoglycemia and with that reduce the secretion of endogenous insulin into the portal system. The rise in systemic insulin levels in the absence of a significant portal-systemic insulin gradient further indicates that insulin, when gradually administered intraperitoneally, finds its route into the systemic circulation not only via the portal circuit but also via direct diffusion from the peritoneal cavity into the systemic circuit. This is indirectly supported by our observations with India ink which, after intraperitoneal infusion in rats, distributes equally over the parietal and visceral peritoneum as well as over the liver lobuli. This may be interpreted to represent the portal drainage from the visceral peritoneum and the systemic drainage not only from the parietal peritoneum but also from the liver lobuli, where absorption is transhepatic (36,37) and, consequently, into the systemic rather than into the portal circuit.

Glucose levels dropped more rapidly with intraportal than with intraperitoneal infusion of insulin, irrespective of the dose infused. With intraperitoneal infusion of 40 and 80 $pmol \cdot l^{-1} \cdot min^{-1}$, severe hypoglycemia was maintained during the complete test period although insulin levels had already normalized. This suggests inadequate counterregulation by glycogenolysis and suppression of glucose utilization (24,40). The prolonged hypoglycemia may well be attributed to a delayed plasma elevation of glucose counterregulating hormones as a consequence of gradual induction of hypoglycemia (40).

Our present observation that intraperitoneally infused insulin is inadequately absorbed appears to contradict our conclusion from a previous study that the peritoneal cavity is a suitable site for an islet graft (41). That study, however, was performed with free, nonencapsulated islet isografts, which are secondarily vascularized. Our present study was undertaken to investigate the suitability of the peritoneal cavity as the recipient site of a bioartificial pancreas, i.e., encapsulated and therefore nonvascularized pancreatic islets. With a 20 $pmol \cdot l^{-1} \cdot min^{-1}$ intraportal insulin infusion, the rise in plasma insulin was similar to that observed in response to a meal test in rat recipients of an intrasplenic and thus portally draining islet isograft of 10 µl, which is the approximate equivalent volume of the native endocrine rat pancreas (42). With this 20 $pmol \cdot l^{-1} \cdot min^{-1}$ intraperitoneal insulin infusion, however, there was no rise in plasma insulin, and glucose levels started to decrease not earlier than 30 min after the start of the infusion.

Apparently, the peritoneal cavity is not a very efficient site for the implantation of an encapsulated and thus nonvascularized islet transplant. One could argue that better insulin responses may be obtained if the volumes of the grafts were larger than the equivalent volume of the native endocrine pancreas, since 40 $pmol \cdot l^{-1} \cdot min^{-1}$ intraperitoneal insulin infusion did induce a rise of plasma insulin levels and a clear-cut reduction of blood glucose levels, and since these effects were even more pronounced with 80 $pmol \cdot l^{-1} \cdot min^{-1}$ intraperitoneal insulin infusion. However, we have previously shown with nonencapsulated islets that a twofold graft volume does not result in a twofold insulin release in response to a glucose challenge (42). Also, an islet graft is known to adapt its activity and mass to the metabolic

demand of the microenvironment (42,43). This, in case of an intraperitoneally located encapsulated islet graft, is the peritoneal fluid. It has been shown that intraperitoneal glucose concentrations in rats follow blood glucose levels with a delay of ~5 min and with a reduction to 80% of blood glucose levels (44). Consequently, the signal to activate insulin release is delayed and reduced, a delay and reduction that may well be enhanced by the presence of the capsules as such (45). Expectedly, therefore, the plasma insulin responses to physiological stimuli in a recipient of any intraperitoneally implanted nonvascularized islet graft will be reduced, irrespective of the graft volume.

Our findings indicate that we should focus on methods to reduce the barriers for glucose to reach the encapsulated islet tissue and for insulin to reach the blood. Apart from modifying the capsules, conceivable approaches are to find or to create a transplantation site that, more than the unmodified peritoneal cavity, permits close contact between the bloodstream and the encapsulated islet tissue. An alternative approach (46,47) may be the stimulation of neovascularization of the capsules.

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