Reduction of Glycemic Potentiation

Sensitive Indicator of β-Cell Loss in Partially Pancreatectomized Dogs

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To determine which test of islet function is the most sensitive indicator of subclinical β-cell loss, we studied six conscious dogs before and 1 and 6 wk after removal of the splenic and uncinate lobes [64 ± 2% pancreatectomy (PX)]. To assess hyperglycemic potentiation, acute insulin secretory responses (AIR) to 5 g i.v. arginine were measured at the fasting plasma glucose (FPG) level after PG was clamped at ~250 mg/dl and after PG was clamped at a maximally potentiating level of 550–650 mg/dl. FPG levels were unaffected by PX (112 ± 4 mg/dl pre-PX vs. 115 ± 5 mg/dl 6 wk after PX, *P* NS). Similarly, basal insulin levels remained constant after PX (11 ± 2 μU/ml pre-PX vs. 11 ± 1 μU/ml 6 wk after PX, *P* NS). The AIR to 300 mg/kg i.v. glucose decreased slightly from 42 ± 9 μU/ml pre-PX to 32 ± 5 μU/ml 6 wk after PX (*P* NS), and thus the β-cell loss was underestimated. In contrast, insulin responses to arginine declined markedly after PX. The AIR to arginine obtained at FPG levels declined from 23 ± 3 μU/ml pre-PX to 13 ± 2 μU/ml 6 wk after PX (*P* = .04). The AIR to arginine obtained at PG levels of ~250 mg/dl declined even more, from a pre-PX value of 56 ± 7 μU/ml to 21 ± 4 μU/ml 6 wk after PX (*P* = .02). However, the largest decline in AIR to arginine occurred at PG levels of 550–650 mg/dl (113 ± 13 μU/ml pre-PX vs. 28 ± 7 μU/ml 6 wk after PX, *P* = .001), thus indicating a marked decrease of maximal glycemic potentiation. Basal levels of glucose and insulin and AIRs to glucose and arginine obtained 1 wk after PX were similar to those obtained 6 wk after PX. The second purpose of the study was to explore mechanisms by which compensation of this β-cell loss occurs and by which hyperglycemia is avoided. One possibility is a reduction of glucagon secretion, but immunoreactive glucagon levels and glucagon secretory responses to arginine measured at three PG levels remained unchanged after PX. Similarly, tissue sensitivity to insulin, measured with euglycemic clamps at two elevated insulin levels, and insulin clearance remained unchanged after PX. Thus, the mechanism for maintenance of euglycemia after partial PX in the dog remains unclear. In summary, a reduction in glycemic potentiation of the insulin response to arginine was found to be the best means of detecting the subclinical β-cell loss induced by a two-thirds PX in dogs.
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Recently, there has been much interest in detecting subclinical islet β-cell loss. For example, if immunosuppressive therapy proves to delay or prevent the development of insulin-dependent diabetes mellitus (IDDM), it will become increasingly important to detect early islet abnormalities in predisposed relatives (1). Unfortunately, because progressive and marked β-cell loss may occur before the onset of overt hyperglycemia in IDDM, hyperglycemia itself cannot be used to discern early β-cell loss (2,3). Therefore, in this study, we have experimentally produced a defined β-cell loss by surgically removing approximately two-thirds of the pancreas in dogs and have assessed the ability of several islet function tests to detect this loss. These tests include basal measures of insulin and glycosylated hemoglobin (HbA_{1c}), the first-phase insulin secretory response to intravenous glucose, the insulin response to intravenous arginine, and hyperglycemic potentiation of the insulin response to arginine.

The second major purpose of this study was to explore the mechanism(s) by which compensation for such β-cell loss occurs and by which hyperglycemia is avoided. Possibilities that were addressed include a reduction in glucagon output, an increase in tissue sensitivity to insulin, and a reduction in the clearance of insulin from plasma. To avoid a major resection for which compensation would be ineffective, the degree of pancreatectomy was limited to 60–70%.

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MATERIALS AND METHODS

A total of 35 experiments were performed in six male mongrel dogs weighing 30–35 kg. The dogs were housed in individual cages that included a 5 × 20-ft area for exercise. Animals were fed 0.5 kg/day of dry standard dog chow (Wayne Pro-Mix) and were given unlimited access to water. A licensed veterinarian supervised care of the animals. Appearance of stool was noted daily, and body weight was recorded immediately before each metabolic experiment. No animal developed any evidence of pancreatic exocrine insufficiency; i.e., there was no diarrhea or weight loss. Initial body weight was 31.8 ± 0.4 kg, and the final weight, obtained just before the final metabolic study, was 31.3 ± 0.5 kg (mean ± SE, *P* NS).

Surgical procedures. At least 2 wk before the study began, a thoracotomy was performed to obtain permanent venous access for subsequent experiments. (Previous experiments have demonstrated that, in dogs, right atrial catheters last longer than peripheral catheters placed via cutdowns; unpublished observations). A 16-gauge indwelling Teflon catheter was placed in the right atrium by sterile technique. After the catheter was secured, the opposite end was routed subcutaneously to the interscapular area, where it was externalized. The catheter was stored in the pocket of a permanent jacket and flushed with heparinized saline twice per week. There were no complications from the surgery or catheter placement.

After the baseline metabolic studies were performed, a partial pancreatectomy was performed during endotracheal intubation with 0.8% halothane/35% nitrous oxide anesthesia. The splenic (left) pancreatic lobe was removed first; then the uncinate process was removed. Special care was taken to place multiple, full-thickness, interrupted, permanent sutures through each stump of the pancreatic remnant to prevent pancreatic enzyme leakage. The duodenal pancreatic lobe and the accompanying two major pancreatic ducts were preserved in all dogs. Dimensions of removed and remaining pancreatic lobes were measured, and the volumes of the removed lobes were subtracted from the total pancreatic volume to provide an estimate of the degree of pancreatectomy. The amount of removed pancreas averaged 64 ± 2% (mean ± SE, range 55–71%). Multiple slices of the splenic and uncinate lobes were preserved in Bouin's solution for 24–48 h, then embedded in paraffin. Sections 20 μm thick were made with a microtome and stained with hematoxylin and eosin for comparison with the pancreatic remnant after all experiments were completed. After surgery, animals received nothing orally for 12 h, then were advanced to a full solid diet over the next 3 days.

When postpancreatectomy metabolic studies were completed, animals were euthanized by an intravenous injection of pentobarbital sodium, and an autopsy was performed. Suture sites were inspected and found to be intact. Multiple biopsies of the duodenal remnant were fixed and stained as before and compared with samples from the removed splenic and uncinate lobes. These remnant slides were examined under light microscopy and found to have no evidence of pancreatitis (i.e., inflammation, cellular destruction, or fibrosis).

One animal, which had previously appeared well, died suddenly 6 wk after surgery; therefore, the final experiment,

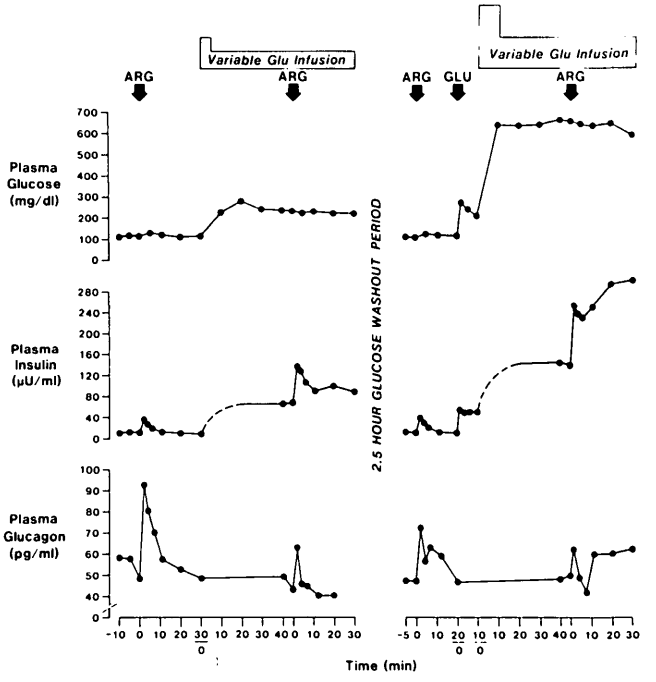

FIG. 1. Summary of islet-function study showing mean plasma values for glucose, insulin, and glucagon at baseline (prepancreatectomy). Insulin and glucagon secretory responses to arginine were measured at fasting glucose level and after hyperglycemic clamp at 200–300 mg/dl. After glucose-washout period, arginine and glucose challenges were given at euglycemia. Finally, insulin and glucagon responses to arginine were obtained after glucose level was clamped at 550–650 mg/dl.

the animal's third insulin-action study, could not be performed. Autopsy revealed a firm ileal adhesion accompanied by a small bowel infarction. The pancreatic bed and sutures were intact.

Islet-function studies. Each animal underwent three islet-function experiments. The first was 20 ± 6 days before the pancreatectomy, and the second and third were 9 ± 1 and 36 ± 4 days after the pancreatectomy, respectively. All experiments were initiated after a 20-h fast and performed when the animal was conscious and resting in a Pavlov sling (Chatham, Los Angeles, CA). Drugs and infusions were delivered through the permanently implanted right atrial catheter, and blood samples were obtained through a 2-inch 18-gauge Teflon catheter inserted into a foreleg vein 15 min before blood sampling was initiated. (Because the venous glucose level is slightly less than the arterial glucose level, such a clamp technique slightly underestimates the arterial glucose level to which the pancreas is exposed.) The volume of blood withdrawn was replaced by at least an equal volume of normal saline to avoid hypovolemia. The procedure and hormone values for the first (prepancreatectomy) study are illustrated in Fig. 1. Baseline 4-ml blood samples for measurement of insulin and glucose were drawn into tubes containing EDTA at −10, −5, and 0 min. At the same time points, 3-ml samples for measurement of glucagon were drawn into tubes containing benzamidine, and 2.5-ml samples for measurement of catecholamines were drawn into tubes containing EGTA and glutathione. A baseline sample for measurement of HbA_{1c} was also obtained. At time 0, arginine hydrochloride (5 g, as a 10% solution) was given intrave-

nously over 30 s. Samples for measurement of insulin, glucose, and glucagon were obtained 2, 3, 4, 5, 7, 12, 20, and 30 min after the bolus to measure the acute insulin and glucagon secretory responses to arginine. Then a primed continuous variable-rate infusion of 20% glucose was begun with a volumetric screw-type reciprocating pump (Harvard model 956, Millis, MA) to raise and maintain the plasma glucose level at ~250 mg/dl. Every 10 min, a blood sample was analyzed on-line for glucose by a technician with a glucose analyzer (Beckman, Brea, CA), and the infusion rate was adjusted by a modification of a previously described algorithm to maintain the glucose level at the desired concentration (4). Forty-five minutes after this infusion began and after prestimulus samples for measurement of insulin, glucose, and glucagon were obtained, a second 5-g arginine bolus was given, and samples from 2 to 30 min were obtained as before. This was followed by a 2.5-h glucose-washout period, during which the plasma glucose was allowed to return to normal to minimize any priming of α- and β-cells from exposure to hyperglycemia (5,6). To rule out priming, a third arginine bolus was given at euglycemia after the glucose-washout period for comparison with the initial insulin response. This insulin response was measured from 2 to 20 min. After additional prestimulus samples were obtained, a 0.3-g/kg bolus of glucose was given over 30 s, and blood samples for measurement of glucose and insulin were obtained 2, 3, 4, 5, 7, and 10 min after delivery to measure the first-phase insulin secretory response to intravenous glucose and to serve as a loading dose for the final hyperglycemic clamp. The plasma glucose level was then raised to 550–650 mg/dl to measure maximal glycemic potentiation of the insulin response to arginine (7). Forty-five minutes after this final glucose infusion began and after prestimulus samples for glucose, insulin, and glucagon were obtained, a final 5-g bolus of arginine was given, and samples were obtained 2–30 min after the bolus as before.

Insulin action. Each animal underwent three insulin-action experiments. The purpose of each was to measure tissue sensitivity to insulin by euglycemic clamps at two elevated but physiologic insulin levels (8,9) and to measure insulin clearance. The first study was performed 12 ± 4 days before the pancreatectomy, and the second and third were 9 ± 1 and 47 ± 2 days after the pancreatectomy, respectively. The second insulin-action study was separated from the second islet-function study by 1–2 days. It was performed after the islet-function study in four dogs and before in two. Duration of fasting, location of infusion and withdrawal catheters, and use of the Pavlov sling for insulin-action studies were the same as in the islet-function studies.

Baseline samples for measurement of insulin and glucose were obtained at −15, −5, and 0 min. A priming dose of regular purified pork insulin (Actrapid, Novo, Wilton, CT) was then given at time 0 at a rate of 2.0 mU · kg^{-1} · min^{-1} for 3 min, followed by a low-dose maintenance infusion at a rate of 0.42 mU · kg^{-1} · min^{-1}. Every 10 min, a 1-ml sample was analyzed on-line for plasma glucose level and a variable-rate infusion of 20% dextrose (to which 10 meq/L of KCl had been added) was adjusted as described to maintain the glucose level at the fasting concentration (4). Blood samples (4 ml) were also obtained at 10 and 40 min and every 10 min from 70 to 150 min for later measurement of plasma insulin and plasma glucose. One hundred fifty minutes after the lower-dose infusion began, a higher-dose insulin infusion was begun. A priming dose of 6.5 mU · kg^{-1} · min^{-1} was given for 2.5 min and was followed by a maintenance rate of 1.30 mU · kg^{-1} · min^{-1}. Blood sampling and adjustment of the 20% glucose infusion were performed as before to maintain euglycemia for an additional 150 min.

Analytical methods. Plasma glucose was measured with a glucose oxidase method (Technicon, Tarrytown, NY). HbA_{1c} was determined by a colorimetric assay (10) based on the thiobarbituric acid test of Fluckiger and Winterhalter (11). Plasma insulin was assayed by a modification of the double-antibody method of Morgan and Lazarow (12). Plasma glucagon was measured by radioimmunoassay with an antiserum of high specificity for the COOH-terminal portion of the pancreatic glucagon molecule (13). Plasma, epinephrine, and norepinephrine were measured by a single-isotope enzymatic assay (14).

Calculations and statistics. Acute insulin secretory responses (AIRs) to arginine and glucose were calculated as the mean of the 2- to 5-min insulin values minus the mean of the prestimulus values.

An estimate of tissue sensitivity to insulin, the insulin sensitivity index (S_I), was calculated as previously described (8,9). S_I equals the increase in the glucose infusion rate (GIR) in milligrams per minute divided by unit increase in immunoreactive insulin level (IRI) during maintenance of constant glycemia. Because the slope ΔGIR/ΔIRI is proportional to plasma glucose level (PG), it is divided by PG to yield an index that is glucose-level independent

$$S_I = \frac{\Delta GIR/kg}{\text{mean PG} \cdot \Delta IRI}$$

Mean values obtained over the final 60 min of the low- and high-dose infusions were used for these calculations.

Insulin clearance was calculated as the mean high-dose insulin infusion rate during the final 60 min divided by the corresponding mean insulin level. Because of the elevated insulin levels, the lack of hyperglycemia, and the effect of exogenous insulin to inhibit endogenous insulin secretion (15), the contribution of endogenous insulin to this insulin level is assumed to be very small.

Student's paired two-tailed *t* test was used to compare differences. Results are presented as means ± SE.

RESULTS

Islet β-cell function. Fasting plasma glucose (FPG) levels were unaffected by a partial pancreatectomy. FPG averaged 112 ± 4 mg/dl before pancreatectomy and 112 ± 5 and 115 ± 5 mg/dl (*P* NS) 1 and 6 wk after pancreatectomy, respectively. FPG levels on insulin-action study days were also similar before and after surgery. In addition, HbA_{1c} levels failed to rise after partial pancreatectomy (4.23 ± 0.4% before surgery and 3.78 ± 0.5% 6 wk after surgery, *P* NS).

Despite removal of two-thirds of the pancreas, basal insulin levels remained at or near baseline levels after surgery. Basal insulin averaged 11 ± 2 μU/ml before pancreatectomy and 8 ± 1 and 11 ± 1 μU/ml (*P* NS) 1 and 6 wk after pancreatectomy, respectively. Insulin values on insulin-action study days were also similar before and after surgery.

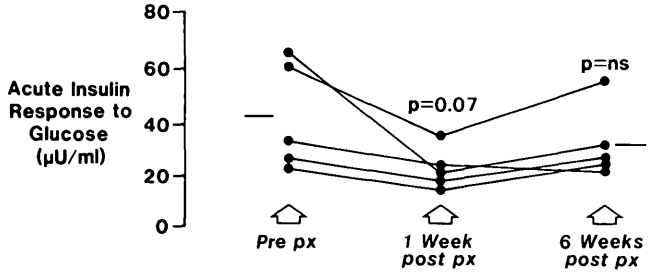

FIG. 2. Individual acute insulin secretory responses to 300 mg/kg i.v. glucose obtained before and 1 and 6 wk after 2/3 pancreatectomy (PX) in conscious dogs (n = 5).

First-phase insulin secretory responses to intravenous glucose are depicted for each animal in Fig. 2. This response averaged 42 ± 9 μU/ml before pancreatectomy and showed a tendency to decline when measured 1 wk after pancreatectomy (24 ± 3 μU/ml, P = .07). When measured 6 wk after pancreatectomy, the first-phase response to glucose was only slightly below baseline (32 ± 5 μU/ml, P NS).

After circulating glucose levels were clamped for 45 min at 200–300 and 550–650 mg/dl, the steady-state insulin levels were measured as an index of the second-phase insulin response to glucose. In contrast to basal insulin, second-phase insulin levels obtained at 200–300 mg/dl were reduced after pancreatectomy (prepancreatectomy, 68 ± 8 μU/ml; 1 wk after, 42 ± 5 μU/ml, P = .02; 6 wk after, 47 ± 7 μU/ml, P = .01). Second-phase insulin levels measured at glucose levels of 550–650 mg/dl were reduced to a similar extent (prepancreatectomy, 152 ± 2 μU/ml; 1 wk after, 76 ± 12 μU/ml; P = .03; 6 wk after, 88 ± 10 μU/ml; P = .04).

The magnitude of the AIR to arginine plotted as a function of plasma glucose level is illustrated in Fig. 3, and individual AIRs are shown in Fig. 4. AIRs obtained at euglycemia declined from a baseline level of 23 ± 3 μU/ml to 11 ± 2 μU/ml 1 wk after pancreatectomy (P = .02) and remained at 13 ± 2 μU/ml 6 wk after pancreatectomy (P = .04). (AIRs obtained at euglycemia before vs. after the glucose-washout period were virtually identical, indicating the absence of priming of the second response by previous hyperglycemia; data not shown.) AIR obtained at glucose levels of 200–300 mg/dl declined even more, from a prepancreatectomy level of 56 ± 7 μU/ml to 19 ± 5 (P = .002) and 21 ± 4 μU/ml (P = .02) 1 and 6 wk after pancreatectomy, respectively. However, the largest decline was seen at glucose levels of 550–650 mg/dl. At these levels the AIR to arginine declined from a baseline value of 113 ± 13 μU/ml to 15 ± 7 μU/ml 1 wk after pancreatectomy (P = .0002) and recovered only slightly to 28 ± 7 μU/ml 6 wk after pancreatectomy (P = .001). In all animals, AIR to arginine at glucose levels of 550–650 mg/dl were lower at both 1 and 6 wk after pancreatectomy than at baseline.

Catecholamine levels did not undergo significant change during the study. Norepinephrine averaged 208 ± 41 pg/ml before pancreatectomy and 185 ± 34 and 246 ± 52 pg/ml (P NS) 1 and 6 wk after pancreatectomy. Similarly, epinephrine levels remained relatively constant (baseline, 137 ± 39 pg/ml; 1 wk after pancreatectomy, 134 ± 32 pg/ml, P NS; 6 wk after, 193 ± 31 pg/ml, P NS). These data do not suggest that any changes in β-cell function were caused by changes in catecholamine levels or sympathetic neural outflow.

Islet α-cell function. α-Cell function was measured to assess whether a reduction in glucagon secretion may have been a means by which animals compensated for a reduced β-cell mass and preserved euglycemia. Although minor changes in glucagon secretion were seen, none was large or consistent. Immunoreactive glucagon (IRG) levels obtained at euglycemia averaged 54 ± 6 pg/ml before pancreatectomy and 42 ± 7 and 64 ± 7 pg/ml (P NS) 1 and 6 wk after pancreatectomy. Similarly, there were no significant changes after pancreatectomy in IRG levels obtained at the two higher glucose levels or in acute glucagon responses to arginine at any of the three glucose levels (data not shown).

Insulin action. S_I averaged 0.34 ± 0.10 ml · kg^{-1} · min^{-1} per μU/ml before pancreatectomy and declined slightly but nonsignificantly when measured 1 and 6 wk after pancreatectomy (0.19 ± 0.6 and 0.21 ± 0.02, respectively). In Fig. 5, glucose infusion rates measured before and 6 wk after pancreatectomy are illustrated as a function of insulin level.

Insulin clearance was unaffected by partial pancreatectomy. Mean values were 21 ± 3 ml · kg^{-1} · min^{-1} before pancreatectomy and 19 ± 4 and 18 ± 2 (P NS) 1 and 6 wk after pancreatectomy.

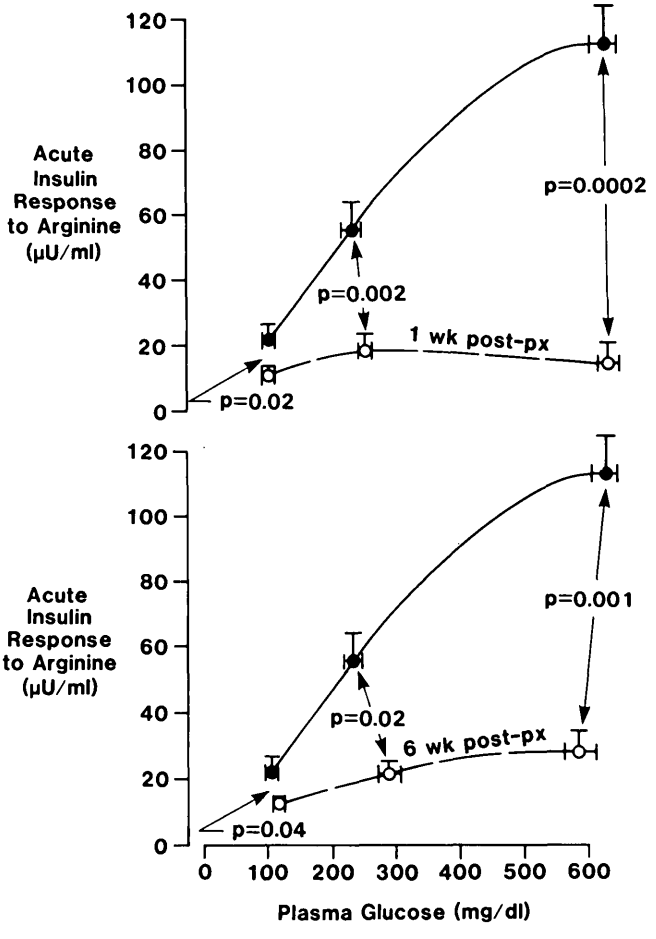

FIG. 3. Acute insulin secretory responses to 5 g i.v. arginine plotted as function of plasma glucose level. Values (mean ± SE) were obtained in conscious dogs (n = 6) before (●) and 1 and 6 wk after (○) 2/3 pancreatectomy (PX).

DISCUSSION

The first major purpose of this study was to assess the effectiveness of several commonly used islet tests in detecting the β-cell loss created by a two-thirds pancreatectomy in dogs. Basal measures of insulin, glucose, and HbA_{1c} failed to detect this β-cell loss. The first-phase insulin secretory response to intravenous glucose underwent a downward trend of borderline significance after pancreatectomy, and by 6 wk it was just slightly and nonsignificantly below baseline. Because the study was small, this decline may have reached statistical significance if more animals had been studied. The finding that removal of two-thirds of the pancreas did not commensurately reduce the first-phase insulin response to glucose has possible relevance to IDDM and non-insulin-dependent diabetes mellitus (NIDDM) in humans. For example, it has been reported that a prolonged prediabetic period exists before the onset of overt hyperglycemia in IDDM, during which the first-phase insulin response to intravenous glucose declines progressively (2,3). Data from the present study suggest that, after a moderate degree of β-cell loss, glycemic potentiation is reduced more than the first-phase insulin response to glucose. Thus, we would predict that during the progressive β-cell loss that may eventually produce overt IDDM, glycemic potentiation would decline sooner than the insulin response to glucose.

The reason that glucose potentiation was reduced more than the AIR to glucose is unclear. It is possible that whereas normal glucose potentiation may require ongoing secretion from a large volume of β-cells, a reduced volume of β-cells may maintain a nearly normal response to glucose by prior packaging of insulin. Although it is known that glucose potentiation is markedly affected by changes in α-adrenergic stimulation (16), we found no evidence of such a change. (Norepinephrine and epinephrine levels after surgery were similar to those before surgery.) Thus, we cannot explain the disproportionate reduction in glucose potentiation by any change in sympathetic neural outflow.

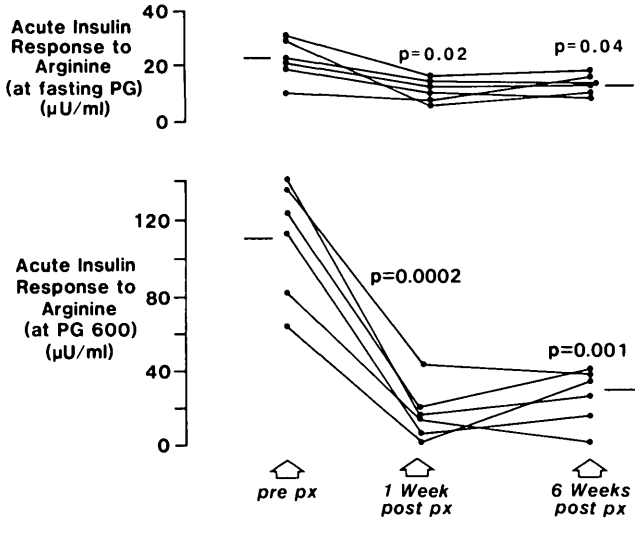

FIG. 4. Comparison of individual acute insulin secretory responses to arginine obtained at fasting glucose levels and at ~600 mg/dl glucose before and 1 and 6 wk after 2/3 pancreatectomy (PX). Note larger postpancreatectomy decline in responses obtained at 600 mg/dl plasma glucose (PG).

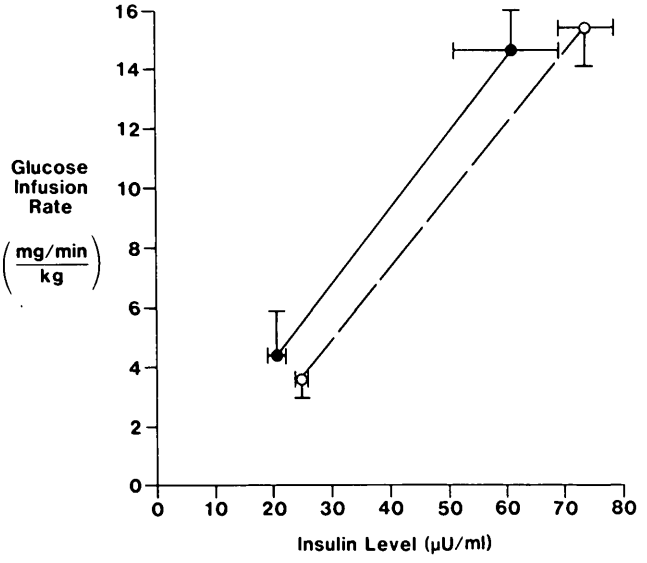

FIG. 5. Comparison of tissue sensitivity to insulin before (●) and 6 wk after (○) 2/3 pancreatectomy. Glucose infusion rates required to maintain euglycemia are plotted as function of insulin levels achieved during 2 hyperinsulinemic clamps. No significant change in insulin sensitivity index (S_I = 0.34 ± 0.10 ml · kg^{-1} · min^{-1} per μU/ml before, S_I = 0.21 ± 0.02 ml · kg^{-1} · min^{-1} per μU/ml after). *n* = 5.

This study suggests that insulin secretion abnormalities in NIDDM are not simply due to a reduced islet mass. In NIDDM the first-phase insulin response to glucose is absent (17). However, interesting reports from Vague and Moulin (18) and Turner et al. (19) suggest that because insulin therapy improved the first-phase response to glucose in NIDDM patients, chronic hyperglycemia may be the cause of this abnormality. In addition, islets cultured at high glucose concentrations have been shown to reduce insulin responses to acute glucose challenges (20,21). The finding in our study that a two-thirds pancreatectomy, a degree of β-cell loss similar to or greater than in NIDDM, is insufficient to abolish the first-phase insulin response to glucose and the findings of these other studies suggest that either a greater degree of pancreatectomy or an additional factor (e.g., chronic hyperglycemia) is needed to abolish this response.

In contrast to the first-phase insulin response to glucose, the AIR to arginine at three glucose levels consistently declined after pancreatectomy. When measured at euglycemia, the AIR to arginine was reduced by ~50% after pancreatectomy. Even more striking, however, was the loss of the ability of hyperglycemia to potentiate the insulin response to arginine (i.e., loss of the synergistic effect of hyperglycemia and arginine to stimulate insulin secretion). Indeed, the insulin response obtained at glucose levels of 200–300 mg/dl was reduced by ~65% after partial pancreatectomy, and the response obtained at glucose levels of 550–650 mg/dl was reduced by 75–87%. Thus, the insulin response to arginine obtained at a glucose level of >500 mg/dl may be the most sensitive index of β-cell loss and may in fact overestimate it.

Bonner-Weir et al. (22) reported that a 90% pancreatectomy in rats, despite some islet regrowth so that the ultimate degree of pancreatectomy was ~60%, resulted in a reduction both in the first-phase insulin response to glucose and the ability of hyperglycemia to potentiate the insulin response

to arginine (23). This reduction in glycemic potentiation was observed both by Bonner-Weir et al. and us, despite widely varying techniques (perfused rat pancreas vs. in vivo conscious dog). In a preliminary report McCulloch et al. (24) also found a marked reduction of glycemic potentiation of the insulin response to arginine in streptozocin-treated conscious baboons. As in our study in partially pancreatectomized dogs, they observed much less of a reduction of the first-phase insulin response to glucose (24). In addition, the same group has gathered preliminary data showing reduced glycemic potentiation of the insulin response to arginine in siblings of IDDM patients who are islet cell–antibody positive and therefore at risk for future IDDM (25). Taken together, these results indicate that subclinical β-cell loss impairs glycemic potentiation of the insulin response to nonglucose stimuli more than it reduces the direct insulin response to glucose.

Second-phase insulin secretion, like the insulin responses to arginine, is influenced by the circulating glucose level, and the second-phase (prearginine) insulin levels, like the responses to arginine, were reduced after pancreatectomy. Although the degree of reduction was substantially less than the corresponding AIR to arginine, the reduction was nearly as consistent. Thus, it can be seen that performance of hyperglycemic clamps allows the use of two tests to detect β-cell loss: the steady-state (second-phase) insulin level and the AIR to arginine. Based on this study, both tests appear superior as predictors of β-cell loss to basal insulin level and the first-phase insulin response to glucose obtained at euglycemia.

In this study, the volume of removed pancreatic tissue, not the volume of islets or β-cells, was measured. Nonetheless, because the distribution of β-cells throughout the canine pancreas is roughly equal in all three lobes (26), we would presume that the fraction of β-cells removed was also approximately two-thirds.

The second major purpose of this study was to examine the mechanism of compensation for reduced β-cell mass. Such compensation was very effective because neither FPG nor HbA_{1c} rose despite normal food intake and maintenance of body weight. Although one possible means of avoiding hyperglycemia after partial pancreatectomy would be a reduction in glucagon secretion, we found no such reduction. Although basal glucagon levels fell slightly (and nonsignificantly) 1 wk after surgery, the mean level was actually slightly higher than baseline 6 wk after surgery. In addition, the acute glucagon response to arginine did not fall significantly after pancreatectomy at any glucose level. This failure of glucagon levels to fall after two-thirds pancreatectomy in dogs is not surprising, because gastric α-cells are a major source of circulating pancreatic-type glucagon in the dog (27).

An increase in tissue sensitivity to insulin could also compensate for β-cell loss. However, this was not found, and in fact, an early trend toward a decrease in insulin sensitivity was noted. This finding suggests that β-cell loss in dogs does not lead to a compensatory increase in insulin sensitivity.

Our data also suggest that the metabolic clearance rate of insulin did not change after pancreatectomy. Indeed, the values remained remarkably constant after pancreatectomy compared with baseline values. In contrast, Marincola et al. (28) reported that hepatic clearance of insulin may decrease after an 80% pancreatectomy in dogs. Like us, they found peripheral levels of basal insulin to remain normal after partial pancreatectomy. They hypothesized that because they observed a modest fall in portal-to-peripheral insulin ratios after pancreatectomy, a reduction in insulin clearance may explain the preservation of peripheral insulin levels. The discrepancy in estimates of clearance between their study and ours might be due either to the differences in methods (portal-to-peripheral insulin ratio vs. metabolic clearance rate during insulin infusion) or, less likely, to differences in degree of pancreatectomy (80% vs. 65%). In designing our study, we chose to avoid portal vein insulin measurements because of technical problems such as streamline flow (incomplete mixing of secreted insulin) and because we sought to estimate whole-body clearance of insulin (both hepatic and renal).

Finally, the pancreatic islets themselves may have adapted to β-cell loss. Because the insulin responses to arginine at the lower glucose levels were less impaired than responses obtained at the higher levels, it is possible that the β-cells become more sensitive to the potentiating effects of glucose after pancreatectomy. Although such a hypersensitivity of the remaining β-cells is an attractive hypothesis, it would require comparison of more extensive dose-response data than we were able to obtain in this study. Thus, the exact mechanism of compensation for this β-cell loss remains to be elucidated.

In summary, a reduction in glycemic potentiation of the insulin response to arginine was superior to basal measures and to the first-phase insulin response to glucose in detecting the subclinical β-cell loss induced by a two-thirds pancreatectomy in dogs. Compensation for such a lesion appears not to be mediated by changes in insulin sensitivity, insulin clearance, or glucagon secretion.

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