Glucagon, Catecholamine, and Symptom Responses to Hypoglycemia in Living Donors of Pancreas Segments

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Donors undergoing hemi-pancreatectomy to provide a pancreas segment for transplantation into a relative with type 1 diabetes acquire diminished insulin and glucagon responses to intravenous agonists. Some donors develop diabetes and require treatment for hyperglycemia. They become at risk for hypoglycemia when treatment includes sulfonylureas and insulin. However, no studies assessing the impact of hemi-pancreatectomy in humans on islet α-cell responses to hypoglycemia have been reported. Consequently, we performed stepped hypoglycemic clamps in 7 donors of varying glycemic control and compared their responses to 16 control subjects. Donors and control subjects reached similar nadirs of glycemia (45 ± 3 and 41 ± 1 mg/dl, respectively) during the clamp. The donors had significantly higher mean basal glucagon levels than control subjects (203 ± 27 vs. 135 ± 15 pg/ml; P < 0.03) but did not have significant differences in glucagon responses during the clamp. The donors also had significantly higher mean peak epinephrine responses during the clamp (1,231 ± 134 vs. 730 ± 68 pg/ml; P < 0.002), but there were no statistically significant differences in norepinephrine or symptom responses. The glucose thresholds at which hormonal and symptom responses began were not different. We conclude that although glucagon response to arginine and insulin response to glucose and arginine are diminished after hemi-pancreatectomy, no deficiency in glucagon responses were detected during hypoglycemia. Diabetes 52:1689–1694, 2003

Pancreas transplantation is usually performed with whole organs or segments of pancreas obtained from cadaveric donors. In some instances, however, a living donor will undergo hemi-pancreatectomy to provide a pancreatic segment for a diabetic family member. The organ survival rate and success in restoring normoglycemia in recipients after segmental transplantation is comparable with the rates obtained with whole cadaveric pancreases. For the donor, however, the risk of developing diabetes is genuine, despite fastidious attention to the exclusion criteria for this procedure, including abnormal glucose tolerance, positive islet cell antibodies, <10 years passage since the onset of type 1 diabetes in the recipient, and obesity. Nonetheless, the risk for development of diabetes in the donors is appreciable. Many donors who have undergone hemi-pancreatectomy experience a decrease in oral glucose tolerance and 25% develop mild diabetes by 1 year after surgery (1). Donors as a group, whether diabetic or not, have diminished α- and β-cell responses to intravenous arginine and glucose stimulation (2) and higher circulating proinsulin-to-insulin ratios than they did preoperatively (3). One to two decades beyond hemi-pancreatectomy, the most reliable predictor of who will develop diabetes is the development of obesity rather than family history of diabetes or re-development of diabetes in their recipient pair (4).

Studies of hormonal counter-regulation of hypoglycemia in donors are needed because some who develop abnormal glucose tolerance will go on to develop diabetes with the possibility of needing sulfonylurea and/or insulin-based therapy and thereby become at risk for hypoglycemia. Consequently, we framed the hypothesis that hemi-pancreatectomy would result in diminished glucagon responses and compensatory increases in catecholamine responses during hypoglycemia with no decrease in symptom recognition. The study protocol used a stepped hypoglycemic clamp with measurements of glucagon, epinephrine, norepinephrine, and symptom response at each plateau of glycemia during the clamp.

RESEARCH DESIGN AND METHODS

Seven human donors of pancreatic segments (50% of original pancreas) were admitted to either the University of Washington or the University of Minnesota General Clinical Research Center. No attempt was made to select donors for this study; rather, all available donors were contacted regardless of their HbA1c or fasting glucose levels. Seven donors responded by agreeing to travel from various parts of the U.S. to one of the two study sites to undergo a stepped hypoglycemic clamp. No patient was taking drugs to manage glucose levels except patient no. 6, who was using insulin and rosiglitazone. The control subjects were not recruited specifically for this study but instead were nondiabetic individuals we had previously studied (5,6) over the previous several years. The institutional review boards at each university approved the study protocol, and written consent was obtained from subjects before study entry.

Methods. After an overnight fast and at bed rest, intravenous catheters were placed in arm veins into which 0.45N saline was infused to maintain patency. Venous blood, arterialized by heating the blood withdrawal site with a heating pad, was sampled from one arm while insulin and glucose were infused into the opposite arm. Blood samples were obtained every 5 min for the immediate determination of plasma glucose. After an initial 30-min rest period, recombinant human insulin (Humulin; Eli Lilly, Indianapolis, IN) was infused at 2 mU · kg−1 · min−1. A variable-rate 20% glucose infusion was used to maintain blood glucose concentrations at the target glucose plateaus of 70, 60, 50, and 40 mg/dl for sequential 45-min intervals. Blood samples were obtained during the basal period and at 15-min intervals for the determination of glucagon, epinephrine, and norepinephrine concentrations. Heart rate and blood pres-
sure were monitored throughout the study. The hypoglycemia symptom score was determined using the symptom score of Hoeldtke et al. (7).

**Assays and data analysis.** Blood glucose was measured at the bedside by a glucose autoanalyzer (Beckman Industries, Fullerton, CA). Blood samples were collected in iced test tubes that contained EDTA/Trasylol (500 units/ml) for glucagon and EGTA (090 mg/ml) and glutathione (60 mg/ml) for catecholamines. Samples were rapidly centrifuged, and serum was stored at -70°C for subsequent analysis. Glucagon levels were determined by radioimmunoassay using the method described by Harris et al. (8). Epinephrine levels were determined using the single isotope radioenzymatic assay described by Evans et al. (9). Statistical comparisons were performed by the Fisher’s PLSD means and Mann-Whitney tests.

**RESULTS**

The seven donors had an HbA1c range of 5.1–16.5%, and a post–hemi-pancreatectomy duration range of 5–18 years. Their ages and levels of fasting insulin and BMI were not statistically different from those of the control group, but fasting plasma glucose levels were statistically different (P < 0.002; Table 1). Two of the three subjects with elevated HbA1c levels by the time of this study had the highest BMIs.

**Hormonal and symptom responses.** All data are reported as means ± SE. The glucose nadir during the clamps in the donors was 45 ± 3 mg/dl (range 32–55) versus 41 ± 1 mg/dl (37–46) in the control subjects (Fig. 1, NS).

The peak increase in glucagon in the donors was 82 ± 14 pg/ml (range 35–135) versus 136 ± 35 pg/ml (28–582) in the control subjects (Figs. 2 and 6; P = NS). The basal glucagon level in the donors was significantly greater than that in the control subjects (203 ± 27 vs. 105 ± 15 pg/ml; P < 0.03, Fig. 2).

The peak increase in epinephrine in the donors was 1,231 ± 134 pg/ml (range 730–1,676) versus 730 ± 68 pg/ml (127–1,237) in the control subjects (Figs. 3 and 6; P = 0.002).

The peak increase in norepinephrine in the donors was 535 ± 127 pg/ml (range 120–1,137) versus 340 ± 53 pg/ml (52–649) in the control subjects (Figs. 4 and 6; NS).

The peak symptom response in the donors was 7 ± 1 (range 6–10) versus 7 ± 1 (4–10) in the control subjects (Figs. 5 and 6; NS).

**DISCUSSION**

Based on our previous observations that donors of a hemi-pancreas have diminished insulin and glucagon responses to oral and intravenous stimulation, we hypothesized that they would have diminished β-cell responses to hypoglycemia. We previously reported that β-cell function during glucose and arginine stimulation is diminished (1,2), a consequence that leads to elevation of plasma proinsulin-to-insulin ratios (3). As the ratios are signifi-

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**TABLE 1**

Clinical profile of hemi-pancreatectomized donors

<table>
<thead>
<tr>
<th>Donor</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Years post-op</th>
<th>HbA1c (%)</th>
<th>Fasting glucose (mg/dl)</th>
<th>Fasting insulin (μU/ml)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>F</td>
<td>5</td>
<td>5.1</td>
<td>118</td>
<td>3</td>
<td>26.7</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>F</td>
<td>14</td>
<td>5.6</td>
<td>99</td>
<td>10</td>
<td>26.4</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>M</td>
<td>16</td>
<td>5.6</td>
<td>113</td>
<td>9</td>
<td>29.1</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>M</td>
<td>5</td>
<td>5.9</td>
<td>97</td>
<td>9</td>
<td>25.0</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>F</td>
<td>16</td>
<td>6.7</td>
<td>133</td>
<td>7</td>
<td>29.6</td>
</tr>
<tr>
<td>6</td>
<td>41</td>
<td>M</td>
<td>10</td>
<td>7.2</td>
<td>159</td>
<td>9</td>
<td>35.3</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>M</td>
<td>8</td>
<td>16.5</td>
<td>275</td>
<td>NA</td>
<td>22.4</td>
</tr>
<tr>
<td>Controls</td>
<td>38 ± 3</td>
<td>8M/8F</td>
<td></td>
<td></td>
<td>92 ± 2</td>
<td>19 ± 7</td>
<td>24.9 ± 0.9</td>
</tr>
</tbody>
</table>

Data are means ± SE unless otherwise indicated.

**FIG. 1.** Plasma glucose levels during the stepped hypoglycemic clamp in 7 donors of hemi-pancreatectomy and 16 control subjects. The goal was to reach glucose levels of 70, 60, 50, and 40 mg/dl by 45, 90, 135, and 180 min, respectively. Fasting plasma glucose levels were significantly different (P < 0.002; see RESULTS), but there were no significant differences in glucose levels at each of the 45-min epochs.
cantly greater than those observed in the donors prepancreatectomy and in control subjects, it is likely that they reflect release of insulin granules that have not yet fully matured rather than a genetic defect in insulin processing (3).

In this study we observed no significant difference in the mean glycemic nadirs reached by the two groups of subjects during the clamps. Although the mean basal glucagon level was greater in the donors, the increase in glucagon levels during the clamps was not statistically different. The epinephrine response in the donor group was significantly greater than that observed in the control group, but the norepinephrine response was not. Symptom responses during the clamps were not significantly different. The glucose threshold at which hormonal and symptom responses began was similar in both groups. Thus, it appears that healthy human subjects can undergo hemi-pancreatectomy without developing diminished α-cell responsivity to hypoglycemia.

The lack of difference in the mean glucagon increments during the clamp in this study is surprising because donors have diminished glucagon responses to intravenous arginine and more readily suppress glucagon secretion during intravenous glucose infusion (2). In addition, we have no explanation for the higher basal glucagon levels in the donors. One possibility that might reconcile the observations of diminished glucagon secretion during the arginine stimulation test and of no diminution during the hypoglycemic clamp is that the arginine dose used was a mid-maximal stimulus, whereas the hypoglycemic challenge was maximal. Thus, one might argue that hemi-pancreatectomy leads to diminished sensitivity rather than diminished maximal response of the α-cell. However, this explanation would not seem to hold because the stepped clamp assesses various levels of stimulation of the α-cell, and at no glycemic plateau were the increments over basal glucagon levels in the donors less than those of the control subjects. Another factor that may have played a role in our results was the unexpectedly higher epinephrine responses in the donors. Although β-adrenergic stimulation...
is known to stimulate glucagon release (10), it is not likely that epinephrine contributed to the higher basal glucagon levels in the donors because basal epinephrine levels were essentially equal in the donors and the control subjects. However, one might speculate that the higher epinephrine response in the donors during the clamp represents an adaptive response that assisted the glucagon response (which was arithmetically lower, although not statistically so, in the donors) and also augmented hepatic glucose production. The classic work of Rizza et al. (11) provides a precedent for this suggested hierarchical response.

The outcome of HbA1c levels in our patients was not unexpected given our previous experience (4), although the patient with a value of 16.5% came as a surprise because of the magnitude. As with many of the donors, this patient does not live near Seattle or Minneapolis and therefore receives medical care in his hometown. This points out the need to be as rigorous as possible in monitoring such patients after hemi-pancreatectomy, because the onset of diabetes after surgery can be subtle and go unrecognized by the donor. This patient is currently receiving oral hypoglycemic agents and responding well. This is consistent with the clinical course of other donors we have studied who developed hyperglycemia and have had good clinical responses to oral agents.

Hormonal counterregulation of insulin-induced hypoglycemia has been extensively studied in patients with type 1 diabetes but much less so in patients with type 2 diabetes. Nonetheless, a similarity exists between the data we obtained from hemi-pancreatectomized donors and data previously published from studies in type 2 diabetic patients. Both Shamoon et al. (12) and Meneilly et al. (13) report that type 2 diabetic patients have attenuated glucagon responses and augmented epinephrine responses. This observation led Shamoon et al. (12) to suggest that the increased epinephrine response may represent an attempt by type 2 diabetic patients to accommodate their lower-than-normal glucagon responses. As indicated above,
we think this line of reasoning may also apply to the tendency of hemi-pancreatectomized donors to have lower glucagon and higher epinephrine responses than their control group. It should be noted, however, that not all studies of type 2 diabetic patients have found alterations in glucagon and epinephrine responses during hypoglycemia (14–16), which may reflect the many variables in this patient group, such as level of glycemic control, antecedent hypoglycemia histories, drugs used for treatment, and age. In this context, it may be relevant that the plasma insulin levels in the donors tended to be lower than those in the control subjects. Cryer et al. (17) have suggested the hypothesis that an essential part of intact glucagon release during hypoglycemia is the “switch off” signal from the \( \beta \)-cell, i.e., that the decrease in insulin release during hypoglycemia signals the \( \alpha \)-cell to release glucagon. If this is true, then it may be that the trend toward lower-than-normal insulin levels in some diabetic patients and in our hemi-pancreatectomized donors, and the resultant lesser drop of insulin during hypoglycemia, may provide a weaker signal to the \( \alpha \)-cell and consequently less glucagon secretion (18). This proposition, however, implies that the intra-islet insulin levels in donors are lower than normal, a difficult assertion to evaluate.

Although one might question the wisdom of living relatives donating pancreas segments to diabetic family members, the request to do so is often made by prospective donors. Our position has been that such individuals need to be fully informed about the potentially negative outcomes of this procedure and must meet the inclusion and exclusion criteria that are designed to screen out those individuals with obvious risks of developing diabetes. Recently, inquiries from relatives about donating pancreatic segments have been received more often because of the attention that successful islet transplantation has received (19,20) and the shortage of cadaveric pancreases. In this context, the more completely we understand the metabolic outcomes of hemi-pancreatectomy, as well as establish appropriate metabolic exclusion criteria, the better prepared physicians will be to counsel donors.

In conclusion, performance of stepped hypoglycemic clamps failed to detect abnormalities in the glucagon response in donors of hemi-pancreatectomy. This result stands in contrast with previous information that donors have diminished \( \beta \)-cell responses to glucose and to arginine and diminished \( \alpha \)-cell responses to arginine.

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REFERENCES


