Impaired phasic insulin and amylin secretion in diabetic rats

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Leahy, Jack L., and Mark S. Fineman. Impaired phasic insulin and amylin secretion in diabetic rats. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E457–E462, 1998.—We have proposed that a hyperstimulated insulin secretion causing β-cell degranulation is the basis for the impaired glucose-potentiated insulin secretion in type 2 diabetes (“overworked β-cell”). To confirm this idea, we previously investigated tolbutamide-infused euglycemic rats. Two novel kinds of β-cell dysfunction were observed: altered phasic glucose-potentiated insulin secretion with preferential sparing of the first phase and a raised secreted ratio of amylin to insulin. The current study tested these parameters in 90% (intact β-cell) and 95% (markedly lowered insulin stores) pancreatectomized (Px) diabetic rats. Rats underwent pancreas perfusion 5–6 wk postsurgery. Controls showed nonchanging insulin secretion during a 20-min perfusion of 16.7 mM glucose + 10 mM arginine. In contrast, both Px groups showed an altered phasic pattern, with the first phase being supernormal (for the β-cell mass) but the second phase reduced in tandem with the insulin content. Amylin secretion from control and 90% Px rats paralleled the insulin output, so that the amylin-to-insulin ratio averaged 0.12 ± 0.03% in the controls and 0.16 ± 0.01% in the 90% Px rats over the two secretory phases. In contrast, the amylin-to-insulin ratio in 95% Px rats equaled that of controls during the first phase (0.12 ± 0.1%) but was twice normal during the second phase (0.32 ± 0.4%). These results confirm the validity of the overworked β-cell schema by showing identical β-cell functional defects in Px rats and tolbutamide-infused normoglycemic rats.

pancreas perfusion; partially pancreatectomized rats; experimental models; type 2 diabetes; pancreas hormone content; radioimmunoassay

INSULINOTROPIC NUTRIENTS and gut factors are the main stimuli of meal-induced insulin secretion. Glycemia modulates these insulin responses (termed glucose potentiation), making it an important compensatory system against postprandial hyperglycemia. Glucose potentiation is impaired in type 2 diabetes (19, 34). We have proposed a novel hypothesis based on the study of diabetic rats (“overworked β-cell” hypothesis), whereby a compensatory upregulation of basal insulin secretion leads to a relative depletion of the β-cell insulin stores, impairing glucose-potentiated insulin secretion on that basis (20). Several kinds of evidence support this schema and its relevance for human diabetes. A period of lowered insulin secretion in diabetic rats secondary to the use of diazoxide or fasting paradoxically increased the pancreas insulin content and ensuing glucose-potentiated insulin secretion in an identical fashion (14, 21). Similar findings have been reported in type 2 diabetes in terms of improved insulin secretion after diazoxide (12), fasting (9), and overnight somatostatin infusion (18). Finally, a hyperstimulated insulin secretion leading to depleted insulin stores was reported to cause the β-cell dysfunction in rat β-cells cultured under high glucose conditions (23).

In a previous study, we infused normal rats with large amounts of the insulinotrop agent tolbutamide (normoglycemia was maintained by adding glucose to the infusate) to study β-cell hyperstimulation in the absence of hyperglycemia (15). Pancreas insulin content was lowered 50%. Two unexpected findings form the basis of the current report. The phasic pattern of insulin secretion to 16.7 mM glucose + 10 mM arginine was altered in the tolbutamide rats, in that the first-phase response was unchanged, whereas the second-phase response was lowered in an identical fashion to the insulin content. The second finding regards amylin, the β-cell peptide that makes up the islet amyloid deposits in type 2 diabetes (7). Amylin is colocalized with insulin in β-cell granules, and it is cosecreted (30). The stored and secreted amylin-to-insulin molar ratios were twice normal in the tolbutamide rats. The current study investigated these parameters in partially pancreatectomized (Px) diabetic rats.

MATERIALS AND METHODS

Px rat model. Male Sprague-Dawley rats weighing 100 g underwent a 90 or 95% pancreatectomy (Px) by the method of Bonner-Weir et al. (3). A laparotomy was performed, followed by mobilization of the splenic and duodenal portions of the pancreas. The pancreas was removed by gentle abrasion with cotton applicators. A 90% Px removed all of the tissue except for the portion bordered by the bile duct and duodenal loop, with care taken to leave the small flap attached to the pylorus. A 95% Px also removed the pyloric flap (24). Control rats (shams) underwent laparotomy and pancreas mobilization. Rats were given standard rat chow until pancreas perfusion 5–6 wk postsurgery.

In situ perfused pancreas and insulin content. Body weight and nonfasting plasma glucose values were obtained on the morning of the pancreas perfusion. The perfusion technique has been described elsewhere (35). The perfusate was a Krebs-Ringer bicarbonate buffer, pH 7.4, that contained 4% dextran T-70 and 0.2% BSA fraction V (Sigma, St. Louis, MO). After a 17-min equilibration period, 1-min samples were collected in tubes on ice that contained 8 mg EDTA. A subset of the samples was assayed for amylin (minutes 1–3 and 15–20 of the perfusion with 16.7 mM glucose + 10 mM arginine), and these tubes contained a cocktail of protease inhibitors (75 µg Elastatinal, 1.5 µg leupeptin, 1.5 mg diso-
tion were calculated by use of minutes 1–3 expressed as means ± SE. Phasic insulin and amylin secretion were calculated by use of minutes 1–3 (first phase) and 15–20 (second phase) of the perfusion with 16.7 mM glucose + 10 mM arginine (see Fig. 2). The method entailed determining the mean secreted hormone concentration for each phase in individual animals and then calculating the values (means ± SE) for the animal groups as a whole. Px values that are expressed as %control were determined by comparing each Px rat to the mean value of the control group and then calculating the values (means ± SE) for all the Px groups. The amylin-to-insulin molar ratio was calculated using the molecular weight for rat amylin of 3,918 and the mean value for rat insulins I and II of 5,767; individual values for each animal were calculated and used to determine the data (means ± SE) for the complete groups. Statistical significance was determined by ANOVA unless otherwise stated.

RESULTS

General characteristics and pancreas hormone contents. Rats were studied 5–6 wk after Px or sham surgery (Table 1). Rats with 90% Px had a nonfasting glycemia level that was 1–2 mM above that of controls (P < 0.04). Pancreas insulin and amylin contents were 39 ± 3 and 43 ± 3%, respectively, of the controls. These values agree closely with the reported 42% relative β-cell mass for these rats 8 wk postsurgery (3). Thus the hormone contents were appropriate for the predicted β-cell mass, and the stored amylin-to-insulin molar ratio was equal to that of the control rats (Fig. 1).

Rats with 95% Px were markedly hyperglycemic (24 mM). Pancreas insulin content was 6 ± 1% of the controls, as opposed to amylin, which was 14 ± 3% of the controls (P < 0.015 by paired t-test). Both results are well below the 42% fractional β-cell mass reported by Montaña et al. (24) for these rats 2 wk postsurgery. Thus these rats were characterized by subnormal hormone contents for the predicted β-cell mass, and the amylin-to-insulin molar ratio was twice normal (P < 0.007).

Phasic insulin secretion. The perfusion protocol and insulin secretion results are shown in Fig. 2. The baseline perfusate was 7.8 mM glucose, followed by 15 min of 16.7 mM glucose and 20 min of 16.7 mM glucose + 10 mM arginine. The sham Px rats showed a biphasic insulin response to high glucose, followed by a fivefold rise in insulin output when arginine was added. The latter response was relatively constant throughout the 20-min perfusion time. Both Px groups showed the expected near-total absence of the insulin response to 16.7 mM glucose. Arginine induced a very different pattern of insulin secretion in the Px groups from the controls. Distinct first and second phases were evident, with both Px groups showing a minimal lowering of the first phase from the controls, as opposed to much lower second phases. The steady-state second-phase response in the 95% Px rats was much below that of the 90% Px rats.

The phasic insulin responses to 16.7 mM glucose + 10 mM arginine (defined as minutes 1–3 for the first phase and minutes 15–20 for the steady-state second phase) are shown in Table 2. In the 90% Px rats, the first-phase and second-phase insulin responses were 79 ± 8 and 37 ± 5% of control vs. the pancreas insulin content from Table 1, which was 39 ± 3% of control. In the 95% Px rats, the results were 50 ± 10 and 7 ± 1% of control vs. a pancreas insulin content of 6 ± 1% of control. Thus there was perfect agreement between the insulin contents and second-phase insulin responses in both groups of Px rats, as opposed to no correlation between the content and first-phase responses.

Table 1. General characteristics of rats

<table>
<thead>
<tr>
<th>Animals</th>
<th>n</th>
<th>Nonfasting Glycemia, mM</th>
<th>Body Weight, g</th>
<th>Pancreas Weight, g</th>
<th>Insulin Content, pmol/pancreas</th>
<th>Amylin Content, pmol/pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Px</td>
<td>5</td>
<td>7.7 ± 0.4</td>
<td>415 ± 9</td>
<td>1.57 ± 0.09</td>
<td>7.9 ± 1.2</td>
<td>59 ± 12</td>
</tr>
<tr>
<td>90% Px</td>
<td>6</td>
<td>9.4 ± 0.6</td>
<td>392 ± 17</td>
<td>0.50 ± 0.03</td>
<td>3.1 ± 0.3</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>95% Px</td>
<td>7</td>
<td>24.1 ± 1.8</td>
<td>352 ± 14</td>
<td>0.41 ± 0.06</td>
<td>0.5 ± 0.1</td>
<td>8 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats/group. Rats were studied 5–6 wk after a sham or partial (90 or 95%) pancreatectomy (Px).
Phasic amylin secretion. Phasic amylin secretion for the 16.7 mM glucose + 10 mM arginine perfusate is depicted in Fig. 3 as sequential (left) and mean (right) amylin-to-insulin molar ratios, with the same time periods used as for the phasic insulin responses (minutes 1–3 and 15–20 for that perfusate, which was minutes 36–38 and 50–55 of the perfusion protocol). In the sham rats, amylin secretion perfectly paralleled insulin release, so that the ratio did not vary over the two phases or among any of the samples (average 0.12 ± 0.03% for all the samples). The same results were obtained in the 90% Px rats (0.16 ± 0.01%, P = nonsignificant vs. sham Px). In contrast, the hormone ratio in the 95% Px rats was equal to that of the controls during the first phase (0.12 ± 0.01%) as opposed to its being twice normal during the second phase (0.32 ± 0.04%, P < 0.003 by ANOVA; P < 0.001 for the first- vs. second-phase values in 95% Px rats by paired t-test).

DISCUSSION

Lowered glucose-potentiated insulin secretion predisposes to postprandial hyperglycemia. The current study provides new insight into this defect in diabetic rats by showing a phasic dichotomy in the insulin response to high glucose + arginine, with the second phase being considerably more impaired than the first phase. This result suggests that different physiological processes mediate the secretory phases. Additional support for differential regulation came from our analysis of the secreted amylin-to-insulin ratios. The second-phase ratio in 95% Px rats was twice normal, but not the first phase, which fits with the suggestion made many years ago by Gold and Grodsky (11) and O'Connor et al. (29) that granule subpopulations exist in β-cells that are stimulus specific. A second observation was a linear correlation between the second-phase glucose-potentiated insulin response and the pancreas insulin content in all groups. That result extends our previous suggestion that the pancreas insulin content exerts a regulatory influence over glucose-potentiated insulin secretion and now focuses that influence to the second phase. In that case, it would be predicted that raising the insulin content in diabetic rats should increase the second-phase but not the first-phase glucose-potentiated insulin secretion. Our fasting study in Px rats corroborated that prediction; the insulin content rose threefold in combination with a tripling of the second-phase insulin response to the incretin glucagon-like peptide-1 + 16.7 mM glucose, whereas the first-phase response was unaffected (14). Thus the main findings in this study, the altered phasic pattern for insulin secretion, the raised amylin-to-insulin ratio, and the correlation between insulin content and second-phase insulin secretion, exactly match findings from our study in tolbutamide-infused normoglycemic rats (15), which provides strong support for our suggestion that a hyperstimulated β-cell is the basis for the defective glucose-potentiated insulin secretion in diabetic rats (20).

This study focused on glucose-potentiated insulin secretion. Is there relevance for glucose-induced insulin secretion in terms of a defective phasic pattern with diabetes? Our perfusion results observed (the expected) near-complete suppression of glucose-induced insulin secretion in both Px groups, with no phasic pattern. Grill et al. (13) showed some years ago that perfusing the pancreas of diabetic rats for 40 min with 0 mM glucose restored glucose-induced insulin secretion. We used this approach in neonatal streptozotocin-diabetic rats and observed a clear difference in the pattern of recovery, with the first phase normalizing but the second phase remaining markedly suppressed (6). In another study, we mapped the evolution of defective glucose-induced insulin secretion in glucose-infused normal rats and observed an intermediate stage, which was characterized by an exaggerated first phase and a suppressed second phase (22). Thus both studies noted phasic patterns for glucose-induced insulin secretion that replicated the glucose potentiation results in this study. We interpret these data to mean that the biochemical basis for the disordered phasic pattern affects both glucose-induced and glucose-potentiated secretion. However, an unidentified β-cell defect suppresses...
glucose-induced insulin secretion, thereby masking the phasic effect.

Analysis of our results against published values for the pancreas \( \beta \)-cell mass in the Px rats provides additional insight into the disordered glucose-potentiated insulin secretion. Rats with 90% Px had a \( \beta \)-cell mass that was 42% of normal 8 wk postsurgery (3). That figure seems valid for this study (5–6 wk postsurgery) because the vast majority of the \( \beta \)-cell regeneration occurs during the first 2 wk (4), and a 40% islet mass was reported 4 wk after the 90% Px (2). Thus the lowered hormone contents and second-phase insulin and amylin secretory responses were attributable to the lowered \( \beta \)-cell mass. In contrast, the first-phase response was 79 ± 8% of control, so that the disordered phasic pattern was secondary to a supernormal first phase rather than to any defect in the second phase. What mediated the increased first phase? While speculative, our hypothesis is increased vagal \( \beta \)-cell stimulation. This idea is based on studies in isolated islets which showed that muscarinic agents potentiated the enteric insulin secretagogues cholecystokinin and gastric inhibitory peptide in a pattern in which the first-phase insulin response was preferentially increased (38). This observation is underscored by the fact that vagal stimulation is linked to the cephalic phase of insulin secretion, which is a rapid, transient increase in insulin secretion that precedes feeding (33). It is thus of interest that increased vagal activity has been noted in hyperglycemic rats (28).

The \( \beta \)-cell mass in 95% Px rats, and thus interpretation of our results, is less clear. A \( \beta \)-cell mass of 42% of normal was reported 2 wk postsurgery (24), but the correctness of that figure for the latter time point in this study is uncertain. Bonner-Weir et al. (3) found a close correlation between the pancreas weight (acinar tissue, because the pancreas is 99% exocrine tissue) and islet \( \beta \)-cell and non-\( \beta \)-cell mass after a 90% Px, suggesting a relatively uniform regeneration of pancreatic tissues. With use of that parameter, the pancreas weight in our 95% Px rats was 26 ± 4% of control, which suggests that the \( \beta \)-cell mass may be somewhat less. Thus whether the 50 ± 10% of normal first-phase insulin response is supernormal or not is uncertain. Regardless, \( \beta \)-cell regeneration is clearly active in these rats, and the main finding in terms of the disordered phasic pattern is the markedly subnormal second-phase response.

Aside from altered phasic insulin secretion, the other major finding in this study was the raised amylin-to-insulin ratio. Although not widely recognized, several papers have reported a raised serum amylin-to-insulin ratio in diabetic rats (10, 16) and type 2 diabetics (5, 17). Importantly, in this study, the ratio was increased only in the 95% Px rats. Glycemia per se is unlikely to be the crucial factor, because the amylin-to-insulin ratio (both stored and secreted) was raised in tolbutamide-infused rats that were normoglycemic (15). Instead, we postulate that the lowered insulin content per \( \beta \)-cell in the 95% Px and tolbutamide-infused rats, but not in the 90% Px rats, is the key finding. A feature that is shared by the 95% Px and tolbutamide rats is suppressed proinsulin synthesis, on the basis of the marked hyperglycemia in the Px rats inhibiting insulin promoter factor-1 expression (37) and inhibition by tolbutamide (8, 31, 32). The milder hyperglycemia in 90% Px rats was instead associated with increased proinsulin synthesis (14). We speculate that the impaired proinsulin synthesis produced granules that were insulin depleted, which would account, in part, for the falling insulin responses. Furthermore, we postulate on the basis of studies that have shown differential regulation of amylin and proinsulin synthesis (25–27) that amylin synthesis is unaffected, or less affected, explaining the raised stored and secreted amylin-to-insulin ratios. However, still needing an explanation is the unchanged amylin-to-insulin ratio for the first-phase response in the 95% Px rats.

In summary, our results provide a temporal sequence for the defective glucose-potentiated insulin secretion...
in P×r rats. With very mild diabetes (when insulin stores are relatively intact), the phasic pattern is disordered because of an exaggerated first-phase response. The second phase at this stage is unaffected and thus reflects the relative β-cell mass. As hyperglycemia becomes more overt, the β-cell insulin stores fall, perhaps because of defective proinsulin synthesis. Granules become insulin depleted, which is manifest as a fall in insulin secretion and a raised circulating ratio of amylin to insulin. This study bolsters the contention in insulin secretion and a raised circulating ratio of amylin to insulin. The authors acknowledge the expert technical help of Peter Nevin.

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