Phenotyping of Individual Pancreatic Islets Locates Genetic Defects in Stimulus Secretion Coupling to Niddm1i Within the Major Diabetes Locus in GK Rats

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The major diabetes quantitative trait locus (Niddm1), which segregates in crosses between GK rats affected with spontaneous type 2-like diabetes and normoglycemic F344 rats, encodes at least two different diabetes susceptibility genes. Congenic strains for the two subloci (Niddm1f and Niddm1i) have been generated by transfer of GK alleles onto the genome of F344 rats. Whereas the Niddm1f phenotype implicated insulin resistance, the Niddm1i phenotype displayed diabetes related to insulin deficiency. Individual islets from 16-week-old congenic rats were characterized for insulin release and oxygen tension (pO₂). In the presence of 3 mmol/l glucose, insulin release from Niddm1f and Niddm1i islets was ~5 pmol . g⁻¹ . s⁻¹ and pO₂ was 120 mmHg. Similar recordings were obtained from GK and F344 islets. When glucose was raised to 11 mmol/l, insulin release increased significantly in Niddm1f and F344 islets but was essentially unchanged in islets from GK and Niddm1i. The high glucose concentration lowered pO₂ to the same extent in islets from all strains. Addition of 1 mmol/l tolbutamide to the perifusion medium further increased pulsatile insulin release threefold in all islets. The pulse frequency was ~0.4 min⁻¹. α-Ketoisocaprate (11 mmol/l) alone increased pulsatile insulin release eightfold in islets from Niddm1f, Niddm1i, and control F344 rats but had no effect on insulin release from GK islets. These secretory patterns in response to α-ketoisocaprate were paralleled by reduction of pO₂ in Niddm1f, Niddm1i, and control F344 rats and no change of pO₂ in GK islets. The results demonstrate that Niddm1i carries alleles of gene(s) that reduce glucose-induced insulin release and that are amenable to molecular identification by genetic fine mapping. Diabetes 50:2737–2743, 2001

Type 2 diabetes is a multifactorial disease that is influenced by environmental and genetic factors (1–3). The disease is a heterogeneous metabolic disorder characterized by impairment of insulin secretion from pancreatic β-cells and insulin resistance in peripheral tissues (4,5). For identifying genes and new mechanisms relevant to the pathophysiology of the disease, genetic dissection of animal models has been initiated (6–13). In the GK rat, which has defects in both insulin secretion and action and demonstrates late complications characteristic of the disease (14–16), genetic analysis identified several quantitative trait loci (QTLs), some with genome-wide significance for diabetes-associated phenotypes (6,7). For further characterization of the major GK-diabetes QTL, Niddm1, congenic strains were established by introgression of the Niddm1 locus onto the genome of the normoglycemic F344 rat. By genetic and pathophysiological characterization of the Niddm1 congenic strains, the locus was divided into two subloci, physically separated in the congenic strains Niddm1b and Niddm1i with distinct metabolic phenotypes (17). Both strains displayed postprandial hyperglycemia and reduced insulin action in isolated adipose cells. Relatively early in life, an insulin secretory defect was suggested in Niddm1i by the decreased serum insulin levels despite hyperglycemia after glucose challenge. Niddm1b also displayed a relative insulin secretory defect later in life, but the phenotype of Niddm1b indicated that the primary defect is in insulin action, rather than in insulin secretion (17,18). To further characterize the difference in insulin secretion phenotype between the two subloci of Niddm1, we investigated the kinetics of insulin release and oxygen tension (pO₂). The measurements were performed in islets from Niddm1i and Niddm1f, which is a subline of Niddm1b (18), in response to changes in the concentrations of glucose, tolbutamide, and α-ketoisocaprate (KIC). The results showed a secretory defect in response to glucose linked to the Niddm1i locus.

RESEARCH DESIGN AND METHODS

Materials. Reagents of analytical grade and deionized water were used. Collagenase, HEPES, and bovine serum albumin (BSA; fraction V) were obtained from Boehringer Mannheim (Mannheim, Germany). Tetrathemethylbenzidine, insulin-peroxidasase, and KIC were bought from Sigma (St. Louis, MO). The rat insulin standard was from Novo Nordisk (Bagsvaerd, Denmark). IgG-certified microtiter plates were purchased from Nunc (Roskilde, Den-
TABLE 1

<table>
<thead>
<tr>
<th>Animal</th>
<th>3 mmol/l glucose (pmol · g⁻¹ · s⁻¹)</th>
<th>11 mmol/l glucose (pmol · g⁻¹ · s⁻¹)</th>
<th>11 mmol/l glucose + tolbutamide (pmol · g⁻¹ · s⁻¹)</th>
<th>(osc/min)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>F344</td>
<td>7.4 ± 2.0</td>
<td>19.2 ± 5.8*</td>
<td>50.7 ± 10.1†</td>
<td>0.38 ± 0.02</td>
<td>9</td>
</tr>
<tr>
<td>Niddm1f</td>
<td>5.3 ± 1.5</td>
<td>10.7 ± 2.8*</td>
<td>31.2 ± 5.3†</td>
<td>0.37 ± 0.02</td>
<td>10</td>
</tr>
<tr>
<td>Niddm1i</td>
<td>4.2 ± 1.1</td>
<td>4.6 ± 1.1</td>
<td>13.4 ± 3.4†</td>
<td>0.37 ± 0.03</td>
<td>9</td>
</tr>
<tr>
<td>GK</td>
<td>8.4 ± 2.2</td>
<td>10.1 ± 2.9</td>
<td>31.0 ± 10.6*</td>
<td>0.39 ± 0.02</td>
<td>9</td>
</tr>
</tbody>
</table>

Data are means ± SE for n animals. Insulin release was measured from islets isolated from F344, congenic strains Niddm1f and Niddm1i, and GK rats. The islets were cultured at 5.5 mmol/l glucose overnight before perfusion in the presence of 3 and 11 mmol/l glucose and 11 mmol/l glucose plus 11 mmol/l tolbutamide. *P < 0.05; †P < 0.01 versus preceding perfusion condition.

Results

Insulin release in response to glucose and tolbutamide. Insulin release from individual islets was measured at 3 and 11 mmol/l glucose (Table 1, Fig. 1). At basal glucose, insulin release was 5.3 ± 1.5 and 4.2 ± 1.1 pmol · g⁻¹ · s⁻¹ from islets isolated from F344 and diabetic GK rats. When the glucose concentration was increased to 11 mmol/l, the rate of insulin release doubled in islets from Niddm1f and normoglycemic F344 rats. In contrast, no change in the insulin secretory rate was observed from Niddm1i and GK islets when the glucose concentration was raised to 11 mmol/l.

The effect of the nonmetabolizable secretagogue tolbutamide on insulin release from islets was evaluated in the presence of 11 mmol/l glucose (Table 1, Fig. 1). The addition of 1 mmol/l of the sulfonylurea to the perfusion medium increased insulin release threefold in islets from all four strains. However, the secretory rates in the presence of 11 mmol/l glucose and tolbutamide differed substantially, with a significantly (P < 0.01) lower absolute rate in Niddm1i islets as compared with F344 islets. Islets from all four strains released insulin in pulses, with frequencies ranging from 0.37 to 0.39 min⁻¹ (Table 1).

Insulin release in response to KIC. The effect of KIC on insulin release from individual islets was assessed in the absence of glucose (Table 2, Fig. 2). In the absence of glucose, insulin release was similar to that observed at 3 mmol/l glucose. Introduction of 11 mmol/l KIC to the perfusion medium caused a prompt rise in insulin release in Niddm1f, Niddm1i, and control F344 islets. In these islets, insulin release increased approximately eightfold during the first 5 min upon exposure to KIC. In contrast, insulin release from GK islets was not affected by KIC.

Oxygen tension in response to glucose. Oxygen tension was measured from isolated islets in the presence of 3 and 11 mmol/l glucose (Table 3, Fig. 3). At low glucose,
pO₂ in islets from congenic Niddm1f and Niddm1i rats was 121.8 ± 3.9 and 125.1 ± 3.0 mmHg, respectively. Similar results were obtained for control and GK islets. Raising the glucose concentration to 11 mmol/l caused a significant decrease of pO₂ by ~6% in islets from Niddm1f, Niddm1i, F344, and GK rats.

**TABLE 2**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Insulin release (pmol·g⁻¹·s⁻¹)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>F344</td>
<td>6.2 ± 1.4</td>
<td>11</td>
</tr>
<tr>
<td>Niddm1f</td>
<td>5.3 ± 1.8</td>
<td>8</td>
</tr>
<tr>
<td>Niddm1i</td>
<td>9.3 ± 2.3</td>
<td>7</td>
</tr>
<tr>
<td>GK</td>
<td>10.0 ± 4.4</td>
<td>7</td>
</tr>
</tbody>
</table>

Data are means ± SE for n animals. Insulin release was measured from islets isolated from F344, congenic strains Niddm1f and Niddm1i, and GK rats. The islets were cultured at 5.5 mmol/l glucose overnight before perfusion in the absence or presence of 11 mmol/l KIC, calculated during the first 5 min. 

Oxygen tension in response to KIC. The effect of KIC on pO₂ in islets from the four rat strains was determined in the absence of glucose (Table 4, Fig. 4). In the absence of glucose, pO₂ was similar to that recorded at 3 mmol/l glucose. In islets from Niddm1f and Niddm1i rats, 11 mmol/l KIC decreased pO₂ by ~15%. Whereas a similar reduction in pO₂ was observed in islets from F344 rats, pO₂ was not significantly decreased in islets from GK rats.

**TABLE 3**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Oxygen tension (mmHg)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>F344</td>
<td>124.3 ± 2.4</td>
<td>10</td>
</tr>
<tr>
<td>Niddm1f</td>
<td>121.8 ± 3.9</td>
<td>6</td>
</tr>
<tr>
<td>Niddm1i</td>
<td>125.1 ± 3.0</td>
<td>9</td>
</tr>
<tr>
<td>GK</td>
<td>120.2 ± 3.2</td>
<td>6</td>
</tr>
</tbody>
</table>

Data are means ± SE for n animals. Oxygen tension was measured from islets isolated from F344, congenic strains Niddm1f and Niddm1i, and GK rats. The islets were cultured at 5.5 mmol/l glucose overnight before perfusion in the presence of 3 and 11 mmol/l glucose. *P < 0.05 versus preceding perfusion condition.
DISCUSSION

The GK rat was established as a diabetes model by selection for offspring with hyperglycemia in successive generations, followed by sister–brother inbreeding to obtain a homozygous strain (23). During the selection process, alleles that contribute to the diabetes phenotype were fixed along several different pathways, e.g., deranged insulin secretion, decreased insulin action in target tissues, dysregulated suppression of hepatic glucose production, or feeding behavior. Exploration of the major diabetes QTL (*Niddm1*) (6) has led to the identification of one series of congenic rat strains that characterizes a sublocus with an insulin-resistance type of diabetes, *Niddm1b/Niddm1f/Niddm1e*. The corresponding congenic strains displayed postprandial hyperglycemia, fasting hyperinsulinemia, increased body weight and fat, reduced lipogenesis in isolated epididymal fat cells, and blunted insulin-stimulated glucose uptake in skeletal muscle (17,18). The disease-causing gene(s) was mapped to a 1-cM region that co-localized with the gene for insulin-degrading enzyme. This region is common to all three congenic rat strains. The GK-derived enzyme encoded two amino acid substitutions that reduced enzyme activity by 30%.

In the present report, we characterized the insulin secretory phenotype of Niddm1f and Niddm1l rats. Congenic rats were therefore studied at the level of individual pancreatic islets by measuring insulin release and oxygen

**FIG. 3.** Oxygen tension measurements from individual islets isolated from control F344 (A), Niddm1f (B), Niddm1l (C), and GK (D) rats perifused in the presence of 3 and 11 mmol/l glucose. Representative experiments of 10 (A), 6 (B), 9 (C), and 6 (D).

**FIG. 4.** Oxygen tension measurements from individual islets isolated from control F344 (A), Niddm1f (B), Niddm1l (C), and GK (D) rats perifused in the absence or presence of 11 mmol/l KIC. Representative experiments of seven (A), four (B), seven (C), and five (D).

**TABLE 4**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Oxygen tension (mmHg)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without KIC</td>
<td>With KIC</td>
</tr>
<tr>
<td>F344</td>
<td>122.6 ± 2.7</td>
<td>108.8 ± 4.5*</td>
</tr>
<tr>
<td>Niddm1f</td>
<td>122.1 ± 5.3</td>
<td>103.4 ± 12.2*</td>
</tr>
<tr>
<td>Niddm1l</td>
<td>125.0 ± 2.8</td>
<td>106.0 ± 6.9*</td>
</tr>
<tr>
<td>GK</td>
<td>128.4 ± 1.9</td>
<td>120.2 ± 3.3</td>
</tr>
</tbody>
</table>

Data are means ± SE for n animals. Oxygen tension was measured from islets isolated from F344, congenic strains Niddm1f and Niddm1l, and GK rats. The islets were cultured at 5.5 mmol/l glucose overnight before perifusion in the absence of glucose for 20 min, after which 11 mmol/l KIC was added. *P < 0.05 versus preceding perifusion condition.
tension under various conditions. The results show that changes in insulin release and oxygen tension in response to glucose, tolbutamide, and KIC in islets from Niddm1f rats are comparable to those in islets isolated from normoglycemic F344 rats. The findings confirm that the diabetes phenotype of the Niddm1f sublocus is not associated with any major defects in insulin secretion.

The other sublocus of Niddm1, Niddm1i, encodes reduced lipogenesis in isolated fat cells and a recessive postprandial hyperglycemia associated with whole-body postprandial serum insulin deficiency (17). Congenic Niddm1i rats contain a 22-cM GK-derived chromosome region that is not contained in Niddm1b (17). Our present results, which show that islets from Niddm1i congenic rats are unable to respond with increased insulin secretion rate to a rise in glucose concentration from 3 to 11 mmol/L, strongly support the notion that Niddm1i encodes a defect in insulin secretion. Indeed, in ≈50% of the Niddm1i islets, the increase in the glucose concentration caused a reduction of insulin release. This phenomenon has been observed in isolated islets as well as in the perfused pancreas from the GK rat (24). This paradoxical insulin response to glucose has been explained by an imbalance between the action of glucose to increase the cytoplasmic Ca2+ concentration ([Ca2+]i) by depolarization and influx of the ion and to decrease [Ca2+]i, by promoting intracellular sequestration and outward transport of the ion (25).

If depolarization is impaired and influx of Ca2+ is reduced, then the lowering effect of glucose on [Ca2+]i will manifest itself in a reduction of insulin release. A transient lowering in plasma insulin is a common observation in patients with type 2 diabetes (26) and may be related to this phenomenon.

The reason for the inability of glucose to stimulate insulin release adequately in the GK rat has been associated with defects in glucose metabolism in the pancreatic β-cell (27,28). Deficient expression of pyruvate dehydrogenase (29), increased secretion of islet amyloid polypeptide (30), alterations in lysosomal enzymes (31), lowered cAMP levels (32), and low levels of mitochondrial glycerol phosphate dehydrogenase (33) all have been suggested in this context. Glycerol phosphate dehydrogenase was mapped to rat chromosome 3 in the vicinity of a region linked to diabetes in GK rats (34). However, overexpression of the enzyme in islets from GK rats was not able to alleviate the impaired glucose-induced insulin secretion (35). Although glucose metabolism may be altered in the GK rat, normal Krebs cycle function has been observed in GK islets (36,37), which is compatible with our observation of a glucose-induced lowering of PO2 in GK and Niddm1i islets. Such an increase in respiration, which is normally coupled to increased insulin release from the islet (22), in combination with the observed secretion impairment, could be explained by decreased coupling between oxidative metabolism and phosphorylation in the β-cells in Niddm1i islets. In support of this view, glucose-induced ATP production was lower in GK islets as compared with control islets (37). A potential candidate for this action is uncoupling protein 2, the expression of which has been demonstrated to affect glucose-stimulated insulin release (38–40).

The secretory defect of the Niddm1i congenic was further characterized by exposing the islets to tolbutamide. This hypoglycemic sulfonylurea acts via closure of the ATP-sensitive K+-channels (41). The stimulatory effect of tolbutamide on Niddm1i islets, although less pronounced than in control F344 islets, indicates that the channels are functional in Niddm1i islets as well as in GK islets, as shown previously (37,42). When the mitochondrial substrate KIC (43) was added to the perfusion medium, insulin release was fully restored in Niddm1i islets. KIC is the deamination product of L-leucine, a reaction catalyzed by branched-chain 2-ketoacid dehydrogenase (BCKDH). The ketoacid is then directly metabolized in the β-cell mitochondria. This finding suggests that the decreased amount of insulin secreted in response to tolbutamide is probably due to insufficient ATP generation (37) for the energy-requiring process of recruiting granules for exocytosis (44,45), rather than decreased insulin content. In GK islets, the leucine deamination product produces a small (46) or, as observed in the present study, almost absent secretory response. This may be related to reduced generation of acetyl-CoA from KIC as a result of defective decarboxylation of this ketoacid by the mitochondrial BCKDH in GK rats (46). However, because Niddm1i islets respond normally to KIC, the genetic defect for the impaired insulin response to KIC in GK islets is unlikely to be related to Niddm1i and, according to syntenic conservation, the BCKDH genes are not located in the Niddm1i region.

In type 2 diabetes, not only are the plasma insulin levels decreased, but also the release kinetics of the hormone are affected. Regular plasma insulin oscillations, which are present in normal individuals (47), are replaced by an irregular plasma insulin pattern (48). The glucose-lowering effect of insulin depends on the release kinetics of the hormone (49,50). The disturbed plasma hormone pattern observed in patients with type 2 diabetes (48) has been connected with the downregulation of insulin receptors (51). It has been speculated that the disturbed plasma insulin pattern may be caused by loss of pulsatile release from the β-cell (26). The present results, that pulsatile insulin release from control F344 islets is comparable with regard to frequency to that observed from GK and the congenic islets, make this speculation less plausible. Instead, less regular plasma insulin oscillations may be caused by the decrease in glucose-induced insulin release manifested as a reduction of the amplitude of the insulin pulses (52).

In summary, genes that influence stimulus secretion coupling have been located to the chromosome region encompassed by the Niddm1i locus. High-resolution genetic mapping of the locus in combination with whole-animal and islet functional studies will provide unique opportunities to locate and study susceptibility genes involved in the cause of impaired insulin secretion in diabetes.

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REFERENCES


