Molecular and cellular responses of islets during perturbations of glucose homeostasis determined by in situ hybridization histochemistry

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ABSTRACT We have evaluated in situ hybridization histochemistry as a means of estimating simultaneously the level of prohormone mRNA and the dimensions of rat pancreatic islets. Localization of the 27-mer 32P-labeled oligonucleotide probes for rat proinsulin I, glucagon, and prosomatostatin I corresponded with localization of antibodies to the three hormones. In normal rats subjected to chronic hyperglycemic clamping, the density of the proinsulin mRNA signal increased 54%, islet size and number increased ~100%, while proglucagon mRNA signal was reduced 81%. Resection of 50% of the pancreas increased proinsulin mRNA 36% and proglucagon mRNA 500%; islet area doubled and islet number increased 50%. In 150-day-old diabetic ob/ob mice, there was an 18-fold expansion in islet area, a 4-fold increase in islet number, but no increase in insulin gene expression. In insulin-dependent streptozotocin-treated diabetic rats, islet area and number were profoundly reduced; insulin deprivation failed to raise proinsulin mRNA in surviving beta cells above control levels. Proglucagon mRNA was high despite the hyperglycemia but was reduced by insulin within 1 hr, suggesting that insulin regulates glucagon gene expression or is required for its regulation by glucose. In situ hybridization of rat islets provides a valid semiquantitative index of insulin and glucagon biosynthesis and of islet dimensions and reveals that normal but not diabetic islets meet increased insulin demand by increasing both number and biosynthetic activity of beta cells.

Normal islets of Langerhans secrete sufficient insulin to maintain the plasma glucose in the normal range during metabolic stress. This compensatory response could involve either an increase of insulin biosynthesis and secretion or an expansion of beta cell mass or both. Since currently employed techniques do not permit quantitative assessment of these components of the compensatory response in the same pancreas, we have evaluated in situ hybridization histochemistry as a means of estimating simultaneously the molecular mRNA and the cellular responses to increased need for insulin production.

METHODS

Experimental Groups and Designs. Pancreata were obtained from intact or Wistar rats after a 5-day hyperglycemic clamp or partial pancreatectomy from spontaneously hyperglycemic ob/ob mice from insulin-deprived streptozotocin-treated diabetic Wistar rats and from appropriate controls. Hyperglycemia was maintained by infusing 50% (wt/vol) glucose at 51–58 mg/min per kg of body weight through Silastic tubing (Dow) previously implanted in an external jugular vein. After 5 days the pancreas was excised under sodium pentothal anesthesia and was processed for in situ hybridization. Age-matched controls received 5% glucose.

In other Wistar rats, a 50% distal pancreatectomy was performed under sodium pentothal anesthesia (1). Four weeks later the remnant was resected under anesthesia. The proximal half of intact pancreata from normal Wistar rats served as controls.

Pancreata of diabetic ob/ob mice of ages 60 and 150 days were resected under anesthesia and compared with nonobese age-matched controls.

Insulin-dependent diabetes was induced in Wistar rats with streptozotocin (65 mg/kg of body weight; Sigma) injected via the external jugular vein. Mean plasma glucose 2 days later was 416 ± 7 mg/dl. Twice daily subcutaneous injections of 2–6 units of isophane insulin (Eli Lilly) were administered at 8:00 a.m. and 3–7 U at 4:30 p.m. daily. All rats received aggressive insulin treatment for 19 days but their regimes varied thereafter until sacrifice 3 days later. Glucagon was measured by radioimmunoassay (2).

In Situ Hybridization. The sections of pancreas were hybridized in situ with 32P-labeled 27-mer oligonucleotide probes synthesized from sequences of rat cDNA for proinsulin I (3), proglucagon (4), and prosomatostatin I (5). A tritiated poly(U) probe (Amersham) was also used. Probes (5–3') were: proinsulin I, GAG CAG GCC CAG CAG GGG CAG CAA GCG; proglucagon I, GTC ACT GTT GAA TGT GCC CTT ACG TGA; prosomatostatin I, GCA GCC AGC TTT GCG TCC CGG GGC TGG. They were labeled with polynucleotide kinase (New England Biolabs) (6). Specific activities ranged from 2000 to 3000 Ci/mmol (1 Ci = 37 GBq).

Two sections of each pancreas were hybridized with the proinsulin oligonucleotide, and two sections were hybridized with the proglucagon probe as described by Han et al. (7). Prohormone mRNA signal density (μm² of signal above threshold per μm² of cells containing that signal) was quantitated by digitizing dark-field images (10 times) of radioautographs under light microscopy by using a videometric 150 image analyzer (American Innovation, San Diego, CA). Islet area was determined by tracing with the screen cursor the boundaries of every islet cell group containing proinsulin mRNA signal. Control pancreata included in each hybridization run were compared by the Student t test for two groups. Indirect immunofluorescence staining for insulin, glucagon, and somatostatin was performed as described (8).

RESULTS

Specificity of Prohormone Probes. To ascertain the specificity of the proinsulin, proglucagon, and prosomatostatin

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oligonucleotide probes, their distribution in rat islets was compared with that of antibodies to the three hormones localized by immunofluorescence staining. The distribution of the three probes corresponded well with that of the appropriate antibody, both with respect to location in the islet and the relative abundance of islet cells labeled (Fig. 1 A–F). The tritiated poly(U) probe signal was diffusely distributed throughout the pancreas (Fig. 1G).

**Effects of Compensation for Hyperglycemic Clamping on Proinsulin and Proglucagon mRNA and Islet Dimensions in Normal Rats.** To determine if in situ hybridization could detect an effect of increased insulin demand on prohormone mRNA and islet dimensions, rats were subjected to hyperglycemic clamping for 5 days. Blood glucose levels averaged 370 ± 130 mg/dl for the first 2 days, but thereafter levels declined to an average of 212 ± 46 mg/dl despite an increase in glucose infusion rates. This, and the absence of glycosuria, were construed as evidence of a compensatory response. Proinsulin mRNA signal density in the pancreatic head and tail was, respectively, 154% and 53% greater than controls (P

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![Image of pancreas sections](image-url)

**Fig. 1.** Comparison of dark-field photomicrograph of pancreas sections hybridized in situ with the $^{32}$P-labeled 27-mer oligonucleotide probes for proinsulin I (A), proglucagon (C), and prosomatostatin I (E) and immunofluorescence staining for antibodies to insulin (B), glucagon (D), and somatostatin (F). A section was also hybridized with a tritiated poly(U) probe (G). (Bar = 50 μm.)
< 0.01). Proglucagon mRNA was 19% of controls (Table 1; Fig. 2A and B) \( (P < 0.01) \). Endocrine area was more than twice that of the controls \( (P < 0.01) \), and the number of islets was almost double (Table 1) \( (P < 0.01) \).

**Effects of Compensation for Partial Pancreatectomy on Proinsulin and Proglucagon mRNA and Islet Dimensions.** To assess these parameters in compensation for another form of increased insulin need, normal rats were subjected to 50% pancreatectomy. Four weeks postoperatively they were aglycosuric, and glucose levels were only 18 mg/dl above controls—evidence of compensation. Proinsulin mRNA density in the pancreatic remnant was 36% above the control value (Table 1) \( (P < 0.01) \). Proglucagon mRNA density was 5 times the controls in this normally glucagon-poor region of pancreas (9), perhaps to compensate for the loss of the glucagon-rich splenic half. Islet area was twice that of the controls \( (P < 0.01) \), and islet number increased by half of the control value \( (P < 0.01) \).

**Proinsulin and Proglucagon mRNA and Islet Dimensions in Spontaneously Hyperglycemic ob/ob Mice.** To examine those parameters during inadequate compensation for hyperglycemia, pancreata from spontaneously hyperglycemic ob/ob mice were examined. In 60-day-old mice (mean glucose level, 317 ± 107 mg/dl; body weight, 44.3 ± 4.2 g), endocrine area was more than 6 times the control value \( (P < 0.01) \), and the number of islets was increased 2.7 fold \( (P < 0.01) \), but proinsulin mRNA density was only 21% above normoglycemic controls (Table 2) \( (P < 0.05) \). In 150-day-old ob/ob mice (mean glucose, 440 ± 108; body weight, 52.8 ± 6 g), the endocrine area was 18 times the control value \( (P < 0.01) \), and the number of islets was 4 times that of age-matched controls (Table 2 and Fig. 3) \( (P < 0.01) \), but proinsulin mRNA density was 17% below normoglycemic control values. Proglucagon mRNA signal was sparse in all ob/ob mice, probably because of attenuation of the alpha cell rim by the ballooning beta cell mass.

**Proinsulin and Proglucagon mRNA in Hyperglycemic Streptozotocin-Treated Diabetic Rats.** The effects of hyperglycemia induced by insulin-deprivation were studied in streptozoto-
cin-treated rats. There was a profound reduction in endocrine area and in the number and size of islets in all treatment groups (Table 3 and Fig. 2C), but prohormone mRNA density varied with the insulin treatment regime. To maintain eu-

Table 2. Measurements as in Table 1 in pancreata from hyperglycemic ob/ob mice and nonobese control mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Endocrine area, %</th>
<th>Islets per cm²</th>
<th>mRNA signal density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Proinsulin</td>
</tr>
<tr>
<td>Controls (60 day; n = 4)</td>
<td>1.0 ± 0.04</td>
<td>91 ± 4</td>
<td>45.3 ± 3.7</td>
</tr>
<tr>
<td>ob/ob (60 day; n = 4)</td>
<td>6.3 ± 1.9† (+530)</td>
<td>242 ± 108† (+166)</td>
<td>54.6 ± 6.9* (+21)</td>
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<tr>
<td>Controls (150 day; n = 3)</td>
<td>1.16 ± 0.35</td>
<td>86 ± 21</td>
<td>52.1 ± 3.3</td>
</tr>
<tr>
<td>ob/ob (150 day; n = 3)</td>
<td>21.3 ± 12.1† (+1736)</td>
<td>330 ± 70† (+284)</td>
<td>48.3 ± 6.6 (−7)</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM of six pancreatic sections hybridized for insulin and for glucagon. Every islet in a section was counted. Only the distal half of the pancreas (tail) was counted. Numbers in parentheses indicate the percent change from the controls.

*P < 0.05.
†P < 0.01.

The measurements of prohormone mRNA by in situ hybridization also corresponded with direct and indirect estimates of hormone biosynthesis obtained with other tech-

Table 3. Measurements as in Table 1 in pancreata from streptozotocin (SZ)-treated diabetic Wistar rats that were insulin-treated (+), insulin-deprived (−), or insulin-deprived and -repleted (−/+)

<table>
<thead>
<tr>
<th>Group</th>
<th>Endocrine area, %</th>
<th>Islets per cm²</th>
<th>mRNA signal density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Proinsulin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Head</td>
</tr>
<tr>
<td>Normal controls</td>
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<td></td>
<td>0.92 ± 0.4</td>
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<tr>
<td>SZ diabetes (n = 9)</td>
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<td>0.22 ± 0.2</td>
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<tr>
<td>Insulin (+) (n = 3)</td>
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<td>0.22 ± 0.2</td>
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<tr>
<td>Insulin (−) (n = 3)</td>
<td></td>
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<td>0.22 ± 0.2</td>
</tr>
<tr>
<td>Insulin (−/+ ) (n = 3)</td>
<td></td>
<td></td>
<td>0.22 ± 0.2</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM of six pancreatic sections hybridized for insulin and for glucagon. Every islet in a section was counted. Numbers in parentheses indicate percent change from controls.

*P < 0.01.
mRNA density failed to rise above normoglycemic controls. Although the latter may reflect residual damage induced by streptozotocin 4 weeks earlier, the functionally insufficient glucose-stimulated enhancement of proinsulin mRNA in two unrelated forms of diabetes calls for further investigation of regulation of insulin gene expression in other forms of the disease. Second, insulin rapidly reduced the elevated proglucagon mRNA in insulin-deprived streptozotocin-treated diabetic rats, suggesting that normal regulation of glucagon gene expression by glucose is either mediated by insulin or requires insulin.

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