Hyperglycemic athymic nude mice: factors affecting in vitro insulin secretion

ADINA ZEIDLER, PORTIA EDWARDS, JOSE GOLDMAN, SMADAR KORT, WOERNER P. MEEHAN, AND SEYMOUR R. LEVIN

Diabetes, Hypertension, and Nutrition, University of Southern California School of Medicine, Los Angeles 90033; Diabetes Research Laboratory and Research Service, West Los Angeles Veterans Affairs Medical Center, University of California Los Angeles, Los Angeles, California 90024; and Henry Ford Hospital, University of Michigan, Detroit, Michigan 48202

Zeidler, Adina, Portia Edwards, Jose Goldman, Smadar Kort, Woerner P. Meehan, and Seymour R. Levin. Hyperglycemic athymic nude mice: factors affecting in vitro insulin secretion. Am. J. Physiol. 263 (Endocrinol. Metab. 26): E1131–E1133, 1992.—The strain of athymic nude male mice (ANM) developed at the University of Southern California (USC) exhibits spontaneous hyperglycemia and relative hypoinsulinemia in vivo. To investigate factors that influence insulin secretion in this animal model of non-insulin-dependent diabetes mellitus, we utilized the isolated perfused mouse pancreas of the ANM-USC and control BALB/c mice. We compared in vitro glucose-induced insulin secretion in ANM USC and control mice, inhibition of secretion by somatostatin, and variability of insulin secretion over the two-year period it took to complete these experiments. Glucose-induced insulin secretion from the isolated pancreas was biphasic in both ANM-USC and controls. Insulin secretion was quantitatively equal to or greater than control mice, depending on the phase of secretion analyzed and the source of the control mice. In contrast to pancreatic β-cells in control mice, insulin secretion from ANM-USC pancreases was relatively resistant to inhibition of insulin secretion by somatostatin. Variability in insulin secretion over the two years in which these experiments were performed was greater from pancreases of control mice than that observed from pancreases of the ANM USC. The hyperglycemic ANM USC mouse does not demonstrate diminished insulin secretion in vitro yet is relatively hypoinsulinemic in vivo. Thus circulating factors other than somatostatin might contribute to the insulinopenic stage in this animal model.

pancreas; perfusion; glucose; somatostatin; non-insulin-dependent diabetes mellitus

PATHOGENESIS OF non-insulin-dependent diabetes mellitus (NIDDM) may be related to either impaired insulin secretion or action (6–10). Previous studies in the athymic nude mouse—University of Southern California (ANM-USC) model have demonstrated a diabetic syndrome without ketosis proneness, normally appearing pancreatic β-cells, and with inappropriately normal or low circulating insulin levels, beginning from ~6 wk of age in the male ANM-USC (11, 12, 14, 15). Although further studies in the ANM-USC have demonstrated peripheral insulin resistance (13), it appears that this animal model may present deficiencies at the pancreatic islet cell level in vivo, as previously described in human diabetes (7). Thus the ANM-USC may simulate some forms of human NIDDM.

The purpose of this study was to further investigate pancreatic β-cell secretion in the whole isolated perfused pancreas from the hyperglycemic ANM-USC in an attempt to define mechanism(s) of the diabetic state in this animal model. We tested whether insulin secretion, which is relatively reduced in vivo, remains reduced when the pancreas has been removed from the mouse. Furthermore, we studied the inhibitory effect of somatostatin on insulin secretion in the isolated pancreas of the hyperglycemic ANM-USC and control mice since somatostatin-secreting cells have been found to be increased in the pancreas of this animal model (14, 11) and therefore may play a role in inhibiting insulin secretion.

ANIMALS AND METHODS

Experimental animals. ANM originating from the BALB/c background at USC were raised and kept in sterile conditions, as previously described (15). Adult male ANM mice were used for the experiments since only males of this strain develop the diabetic syndrome, whereas normal male BALB/c mice were used as controls. The BALB/c mice were obtained from two sources: a strain raised at USC (during 1982–1987) and from Simonsen Labs (Gilroy, CA, 1988–1989). Both control and experimental animals were fed similar diets of Wayne Sterilized Lab Blox (Applied Mills, Chicago, IL) and were supplied sterilized water. The day before the experiments, animals were placed in separate cages, weighed, and fasted overnight. Throughout the duration of the study, control BALB/c experiments were performed along with ANM-USC. All animals were maintained and used in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all procedures involving animals were approved by the USC Animal Care and Use Committee.

Isolated perfused pancreas experiment. ANM-USC and control mice, 8–10 wk of age (young adults), were fasted overnight and anesthetized with 60 mg/kg intraperitoneal pentobarbital sodium. Experiments were initiated between 10:00 and 12:00 A.M. For isolated perfused mouse pancreases, we used the previously described method (3). This procedure was modified using smaller cannulas to adapt to the pancreatic vasculature of a mouse. Furthermore, instead of perfusion of the pancreas, spleen, and stomach, as in the rat (3), in the mouse the preparation included part of the small intestine as well. Perfusion buffer was Krebs Ringer solution with 0.2% human serum albumin 3% low-molecular-weight Dextran (Pharmacia Laboratories, Piscatawan, NJ), pH 7.4, 37.5°C. Flow rate was 1 ml/min, range 0.6–1.4 ml/min. Perfusate was kept oxygenated with a mixture of 95% O2–5% CO2. Each perfusion experiment utilized a single-pass nonrecycling system. The arterial pressure was kept constant at 10–15 mmHg. Immunoassay of insulin was measured using an antibody-coated tube method as previously described (4) or a double-antibody homologous method (5). Basal glucose concentration was 60 mg/dl, and stimulatory glucose concentration was 300 mg/dl. Cyclic somatostatin for the perfused pancreas experiments was obtained from Peninsula Laboratories.

Statistical analysis. Data were analyzed using the paired or unpaired Student’s t test and by analysis of variance when indicated (1). Summated immunoreactive insulin was obtained by adding individual insulin values measured during a designated secretory period. Data were expressed as means ± SE.
RESULTS

Effects of glucose on insulin secretion in control and ANM-USC mice. Although the ages were similar in ANM-USC and controls (9.0 ± 1.0 vs. 8.0 ± 2.0 wk, \( P = \) not significant), the weight of ANM-USC was higher as compared with control mice (22.1 ± 0.8 vs. 20.8 ± 0.3 g, \( P < 0.02 \)).

In response to 300 mg/dl glucose, insulin secretion was biphasic in both the ANM-USC and control mice (Fig. 1).

There were trends toward greater insulin release in ANM-USC in both secretory phases. Summated insulin secretion during the early period (1-6 min) was 1,959 ± 505 vs. 1,166 ± 198 \( \mu \)U/ml, and, during the second phase (7-47 min), secretion was 12,518 ± 5,581 vs. 6,064 ± 988 \( \mu \)U/ml; however, these differences were not significant. Although neither the first nor second phase differed statistically in either group, peak early release (2-3 min) in ANM-USC was significantly greater when compared with control mice (1,013 ± 266 vs. 645 ± 127 \( \mu \)U/ml, \( P < 0.05 \)) as was insulin release during the earlier portion of the second secretory phase (10-13 min, 989 ± 275 vs. 428 ± 85 \( \mu \)U/ml, \( P < 0.05 \)).

Inhibitory effects of somatostatin on insulin secretion.

Nine male ANM-USC and seven male control mice were used for the experiments. The ages (9.2 ± 0.8 vs. 9.0 ± 1.1 wk) and weights (23.0 ± 1.0 vs. 21.0 ± 0.5 g) were similar in both groups.

In pilot experiments, with a variety of somatostatin concentrations with BALB/c mice, it was found that 10 ng/ml of this peptide inhibited glucose (300 mg/dl)-induced insulin secretion by 50%. Therefore, 10 ng/ml somatostatin was used in these experiments.

After stabilization for 45 min at 60 mg/dl glucose, concentration was increased to 300 mg/dl, which persisted for 35 min. The period of secretion just before somatostatin infusion was introduced (segment A; 10-13 min) was compared with subsequent somatostatin periods. Somatostatin was then superimposed to determine its effect on insulin release. Significant inhibition of secretion by somatostatin was observed in control mice during segment C (20-25 min) and segment D (24-27 min; Table 1). After somatostatin was withdrawn, there appeared to be a postinhibitory accentuation of secretion from the control pancreases but not from the ANM-USC group. Although there was relative inhibition by somatostatin of insulin secretion in the ANM-USC, the difference did not reach significance.

Long-term variability of insulin secretion in ANM-USC and control mice. Insulin secretion from isolated pancreases of control mice taken from two time periods are compared in Table 2. The early group was studied in 1986 through 1987, when mice were obtained from the USC colony of BALB/c mice. Secretion was compared with that of control mice studied in later phases (1988-1989), when mice were obtained from Simonsen Laboratories. Those comparisons are also made for ANM-USC studied in the early vs. later years. Secretion was more variable, in response to the same stimuli, in pancreases from control mice between the two periods, whereas a more consistent secretion of insulin from ANM-USC pancreases was evident throughout the years of this study (Table 2). A significantly different response was seen for summated immunoreactive insulin during second-phase secretion.

Table 1. Effect of somatostatin (10 ng/ml) on summated insulin (\( \Delta IR I, \mu U/ml \)) response to glucose (300 mg/dl) during different experimental periods (segments A-E) in control and ANM-USC

<table>
<thead>
<tr>
<th>Phase</th>
<th>Segment</th>
<th>Control (S) ( (n = 7) )</th>
<th>ANM-USC ( (n = 9) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1,490±2406</td>
<td>698±269</td>
</tr>
<tr>
<td>Pre inhibitory</td>
<td>A ( (minutes 10-13) )</td>
<td>1,234±617</td>
<td>331±97</td>
</tr>
<tr>
<td></td>
<td>B ( (minutes 16-19) )</td>
<td>857±354*</td>
<td>349±93</td>
</tr>
<tr>
<td></td>
<td>C ( (minutes 20-23) )</td>
<td>778±311*</td>
<td>429±135</td>
</tr>
<tr>
<td>(somatostatin in)</td>
<td>D ( (minutes 24-27) )</td>
<td>778±291†</td>
<td>544±176</td>
</tr>
<tr>
<td>Post inhibitory</td>
<td>E ( (minutes 32-35) )</td>
<td>1,664±635</td>
<td>544±176</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; \( n \), no. of mice. ANM-USC, athymic nude mice developed at the University of Southern California; \( \Delta IR I \), immunoreactive insulin; control \( (S) \), BALB/c control mice obtained from Simonsen Labs. * \( P < 0.02 \) (A vs. C), summated insulin secretion during segment C \( (minutes 20-23) \) compared with prehibitory phase segment A \( (minutes 10-13) \) in control mice. † \( P < 0.01 \) (A vs. D), summated insulin secretion during segment D \( (minutes 24-27) \) compared with prehibitory phase segment A \( (minutes 10-13) \) in control mice.

Fig. 1. Glucose-stimulated insulin secretion in athymic nude mice—University of Southern California (USC; \( n = 11 \)) strain and control BALB/c-USC (\( n = 18 \)) mice during whole pancreas-perfused experiment. Data are expressed as means ± SE.
Table 2. Summed immunoreactive insulin levels (2IRI, μU/ml) in isolated perfused pancreas in control BALB/c and ANM-USC during early period and later period

<table>
<thead>
<tr>
<th>Insulin Secretory Phase</th>
<th>Summed IRI, μU/ml</th>
<th>n</th>
<th>First phase (minutes 1-6)</th>
<th>Second phase (minutes 7-22)</th>
<th>Total (minutes 1-22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (USC)</td>
<td></td>
<td>6</td>
<td>671±176</td>
<td>813±204</td>
<td>1,483±423</td>
</tr>
<tr>
<td>Control (S)</td>
<td></td>
<td>10</td>
<td>1,166±198</td>
<td>1,999±209</td>
<td>3,161±232</td>
</tr>
<tr>
<td>ANM-USC</td>
<td></td>
<td>9</td>
<td>1,095±349</td>
<td>2,517±267</td>
<td>3,512±499</td>
</tr>
<tr>
<td>ANM-USC</td>
<td></td>
<td>11</td>
<td>1,959±305</td>
<td>4,345±1,489</td>
<td>6,304±1,979</td>
</tr>
</tbody>
</table>

Data are means ± SE; n no. of mice. Early period, experiments performed during 1986-87; control (USC), BALB/c mice bred and raised at University of Southern California; Later period, experiments performed during 1988-89. * P < 0.05, early vs. late period control mice.

DISCUSSION

This is the first study to assess insulin secretion from the isolated perfused pancreas of ANM mice bred at USC. This animal model simulates human NIDDM in several aspects of carbohydrate metabolism (11-15). We compared pancreas from hyperglycemic male ANM-USC and control BALB/c male mice. In contrast to our previous finding of relatively reduced insulin secretion in vivo, in one set of perfusion experiments, insulin release from the isolated pancreas was significantly higher at specific times in the ANM-USC, as was early release, when compared with controls. The glucose stimulus (300 mg/dl) that we used in these present in vitro studies was quite similar to the levels seen in our previous in vivo work. It is of interest that the insulin secretion evoked by glucose was enhanced in this animal model when compared with the BALB/c-USC controls, resembling observations in early mild NIDDM (7). To test whether ANM-USC mice might have increased sensitivity of the pancreatic β-cells to an inhibitor of secretion, we examined the effects of somatostatin on the isolated pancreas preparation. In contrast to this hypothesis, our data indicate that somatostatin was less effective in initiating secretion in the ANM-USC mice than in control mice. Because our previous studies (11) indicated a significantly increased proportion of pancreatic somatostatin-secreting cells despite a normal number of insulin-secreting cells and normal insulin content in the hyperglycemic ANM pancreata, our observations could imply that there is downregulation of somatostatin receptors, which are located on the β-cell secretory vesicles (2).

Over the period of time these experiments were done, two sources of control mice were examined. Although we found consistent insulin secretory rates from ANM-USC pancreata, there was considerable variation in secretion from control mice. In the early part of these experiments when we used BALB/c (bred at USC) mice, glucose-stimulated insulin release was characteristically less than that of the ANM-USC. However, during the later stages of these studies, glucose-stimulated insulin release was greater in the control BALB/c obtained from another source (Simonsen Labs). This emphasizes that control animals from a similar strain (BALB/c) but from different sources must be monitored periodically for consistency of insulin secretion to validate experimental results.

In summary, insulin secretion was not diminished in vitro from ANM-USC pancreases. This model has been shown to have relatively reduced insulin secretion in vivo. Other circulating inhibitors or increased metabolism of insulin by the liver might be considered in the pathogenesis of the diabetic syndrome in this animal model.

We are thankful for the technical assistance of Clara DelGado. We acknowledge the expert typing of Deborah McCline. We thank Patrick Stucky for editorial assistance and preparation of the manuscript for publication.

This study was supported in part by a grant from the American Diabetes Association, California Affiliate, and by the Louise and Gustavus Pfeiffer Foundation.

Address for reprint requests: A. Zeidler, Los-Angeles County University of Southern California Medical Center, 1200 N. State St., Rm. 8250, Los Angeles, CA 90033.

Received 17 May 1992; accepted in final form 13 July 1992.

REFERENCES