Loss of insulin response to glucose but not arginine during the development of autoimmune diabetes in BB/W rats: Relationships to islet volume and glucose transport rate

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ABSTRACT The insulin and glucagon responses to 10 mM glucose and 10 mM arginine were studied in pancreata isolated from nondiabetic diabetes-prone and diabetes-resistant BB/W rats at 60, 80, and 140 days of age and in diabetic BB/W rats on the 1st and 14th days of their diabetes. In the former group the insulin response to glucose declined progressively with age (r = −0.575; P < 0.01) and at 140 days was significantly below age-matched diabetes-resistant controls (P < 0.05). The insulin response to arginine did not decline with age in either group. For diabetic rats, on the first day of the diabetes, the insulin response to glucose was absent but the response to arginine did not differ from nondiabetic controls. On day 14 responses to glucose and arginine were both absent. The glucagon response to arginine showed no trend despite a decline in baseline glucagon secretion. Endocrine tissue in nondiabetic diabetes-prone rats made up 0.8 ± 0.2% of the pancreas at 60 days of age and 0.52 ± 0.22% at 140 days of age; the latter was significantly less than in 140-day-old diabetes-resistant controls (P < 0.05). In diabetic rats on the 1st and 14th days of diabetes endocrine tissue was 0.2 ± 0.1% and 0.07 ± 0.02%, respectively. The glucose transport rate in islets isolated on the first day of diabetes was profoundly reduced compared to age-matched nondiabetic diabetes-prone controls. Thus, a population of arginine-responsive, glucose-unresponsive islets with low glucose transport rates is present at the onset of overt diabetes in BB/W rats.

Attenuation of the β-cell response to glucose stimulation without impairment in the response to nonglucose secretagogues was first described in human patients with very mild type 2 diabetes (1) and has since been observed in the prediabetic phase of human type 1 diabetes (2). The potential pathogenic significance of a selective early loss of glucose responsiveness in such disorders and its possible relationship to glucose transport prompted these longitudinal studies of islet function, morphology, and glucose transport in prediabetic and diabetic BB/W rats, the most widely employed rodent model of autoimmune-mediated diabetes.

MATERIALS AND METHODS

Diabetes-prone and diabetes-resistant BB/W rats were obtained from the University of Massachusetts at 50 days of age and kept in metabolic cages with free access to standard laboratory chow. Nondiabetic animals from both groups were studied at 60, 80, and 140 days of age, while diabetic rats from the diabetes-prone group were studied on the 1st and 14th days of diabetes (defined as a fasting blood glucose level >199 mg/dl accompanied by glycosuria). Blood glucose levels were determined daily from tail blood samples. Urinary glucose was measured daily using Ketodiastix.

In confirmation of the work of Marliss et al. (3), the daily fasting glucose determination in diabetes-prone rats was generally normal until 48 hr before the appearance of permanent hyperglycemia in excess of 199 mg of glucose per dl. At that time, levels averaged 157 ± 10 mg/dl, significantly (P < 0.001) above the mean (±SD) glucose level of the group as a whole (122 ± 15 mg/dl, n = 907). However, over half of the rats that did not become diabetic also exhibited a transient blood glucose level above 152 mg/dl, which is two SDs above the mean fasting glucose level, indicating that mild hyperglycemia did not necessarily herald the onset of diabetes.

On the day of the study 50 mg of pentobarbital per kg was given i.p., and the rat pancreas was isolated and perfused by the method of Grodsky and Fanska (4) as described in detail (5). Krebs–Ringer bicarbonate buffer containing 5.6 mM glucose and 5 mM each of pyruvate, fumarate, and glutamate was perfused at a flow rate of from 1.8 to 3.6 ml/min in accordance with body weight. Following a 10-min equilibration period effluent perfusate was collected in chilled tubes at 1-min intervals. After a 10-min baseline perfusion period, the glucose concentration was raised to 10 mM for 10 min and then lowered to the original baseline for 5 min. At the end of this "rest" interval 10 mM arginine hydrochloride was perfused for 10 min. After a second 5-min rest interval, 10 mM arginine and 10 mM glucose were perfused in combination for 10 min.

Insulin was measured by the method of Yalow and Berson (6) with modification (7). Glucagon was measured using antibody 30K (8). The hormone "response" to a given stimulus was the average of the 10 values during that 10-min period of stimulation. Data are shown as mean ± SE. The statistical significance of differences was estimated by Student’s t test.

After each perfusion the pancreas was fixed in Bouin’s solution and processed for paraffin embedding. Sections averaging 5 μm were stained with hematoxylin/eosin, with guinea pig anti-insulin antibody, or with rabbit anti-glucagon or anti-somatostatin antibody for indirect immunofluorescence as described (9, 10). The volume density of the islet endocrine tissue vs. the exocrine acinar tissue was determined using the point-counting method (22) on hematoxylin-eosin-stained sections at a magnification of 175×. The results are expressed as percentage of acinar tissue (22).

Uptake of 3-O-methyl-d-glucose was measured in islets isolated by collagenase digestion of pancreata (11). Individual islets were placed in 400-μl polystyrene microfuge tubes containing Krebs-Ringer solution buffered with bicarbonate and 20 mM Hepes, pH 7.4. Assays were routinely performed.

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with 50–100 islets per tube. Following one wash by centrifugation for 30 sec in a Beckman microfuge, islets were resuspended in 100 µl of buffer without glucose and assayed for 3H-labeled 3-O-methyl-d-glucose uptake using the dibutyl phthalate centrifugation method of Gorus et al. (12). Zero-time determinations were made using islets incubated for 15 min at 4°C, and rates of uptake of 3H-labeled 3-O-methyl-d-glucose (20 mM final concentration; 0.1 µCi/µmol; 1 Ci = 37 GBq) were made after a 30-sec incubation at 37°C.

The DNA from islet cells was prepared by the method of Maniatis et al. (13). The washed islets were lysed in 0.05 M EDTA/0.02 M Tris-HCl, pH 8.0, containing proteinase K (400 µg/ml) and 0.5% sodium laurylsarcosine at 50°C for 2–3 hr. The lysate was mixed with Ficoll-400 and bromophenol blue to final concentrations of 2.5% (wt/vol) and 0.4 mg/ml, respectively. At least two dilutions of this solution were subjected to electrophoresis on a 0.3% agarose gel in 0.04 M Tris acetate/0.002 M EDTA, pH 8.0, in the presence of ethidium bromide (0.5 µg/ml). Several different dilutions of salmon sperm DNA solution were also included as standards.

The gel was photographed on an ultraviolet transilluminator using a Polaroid type 55 film. The negative was scanned on a Hoefer scanning densitometer connected to an IBM PCXT. Standard curves were constructed by plotting the area of each standard DNA peak against the amount of DNA in ng. The amount of DNA in islets was calculated from the area of the peaks for these samples.

RESULTS

Response of Insulin and Glucagon to Glucose and Arginine in 60-, 80-, and 140-Day-Old Nondiabetic Diabetes-Prone Rats

The mean glucose level just before the experiment in the diabetes-prone rats did not differ from age-matched diabetes-resistant controls (102 ± 5.1 vs. 95.5 ± 5.9 mg of glucose per dl at 60 days, 93.8 ± 2.8 vs. 88 ± 1.7 mg of glucose per dl at 80 days and 91.4 ± 3.8 vs. 99.4 ± 5.4 mg of glucose per dl at 140 days).

The mean baseline insulin level in perfusate of isolated pancreata declined significantly (P < 0.01) in perfused pancreata of both diabetes-prone and resistant groups between 60 and 140 days [10.7 ± 1.8 micromunits/ml to 3.4 ± 0.5 micromunits/ml in diabetes-prone rats; 14.6 ± 0.3 micromunits/ml to 5.4 ± 1.0 micromunits/ml in the diabetes-resistant group (Fig. 1)]. The mean insulin response to glucose in isolated pancreata from nondiabetic diabetes-prone rats also declined during this period (not significant, NS) [19.8 ± 3.4 micromunits/ml at 60 days of age (Fig. 1; see Fig. 3 Left) and 11.6 ± 1.6 micromunits/ml at 140 days]; the values at 80 and 140 days were significantly below the glucose-induced insulin response of age-matched diabetes-resistant rats (P < 0.05) (Fig. 1) and the responses were negatively correlated with age (r = -0.575; P < 0.01).

In contrast, insulin levels during perfusion with 10 mM arginine did not decline with age, and there were no differences between groups. During perfusion with glucose and arginine together, the insulin response exceeded the sum of the responses to each substance perfused alone (see Fig. 3 Left), and this did not change with age (data not shown).

The mean baseline glucagon level in perfused pancreata of the nondiabetic diabetes-prone group was significantly lower at 140 days than at 60 days (P < 0.01) (Fig. 2). The 140-day value was also significantly below the baseline level of the 140-day-old diabetes-resistant group (P < 0.01), in which there was no change with age (Fig. 2). Changes in glucose-suppressed and arginine-stimulated glucagon did not reveal a trend.

Response of Insulin and Glucagon to Glucose and Arginine on the 1st and 14th Days of Diabetes

On the first day of diabetes the mean ± SEM of plasma glucose levels obtained immediately before each experiment was 233 ± 7 mg/dl. In perfused pancreata from these animals (mean age of 84.8 ± 6.1 days) the baseline insulin level (20.0 ± 4.6 micromunits/ml) was almost twice the level of 60-day-old nondiabetic diabetes-prone animals (NS) (Fig. 1). Insulin did not rise in response to 10 mM glucose (Figs. 1 and 3). In six other studies (data not shown) glucose perfusion at concentrations of 10, 20, and 30 mM also failed to stimulate insulin secretion on the first day of diabetes.) However, during perfusion with...
10 mM arginine, the insulin response (67.1 ± 6.9 microunits/ml) was not significantly different from that of nondiabetic diabetes-prone or resistant rats (Figs. 1 and 3). The mean insulin concentration during combined perfusion with glucose and arginine (71 ± 16.4 microunits/ml) was not significantly greater than with arginine alone (Fig. 3).

Baseline glucagon levels of perfused pancreata on the first day of diabetes (144 ± 23 pg/ml) were significantly lower than in nondiabetic diabetes-prone rats [465 ± 26 pg/ml at 60 days and 261 ± 31 pg/ml at 140 days (P < 0.01 and P < 0.05, respectively)] (Figs. 2 and 3). There was no suppression by glucose on the first day of diabetes. During perfusion with arginine, glucagon concentration was somewhat greater than in nondiabetic controls (NS). The response to combined perfusion with glucose and arginine did not differ from that to arginine alone (Figs. 2 and 3). Fourteen days after the onset of diabetes the mean baseline glucagon level had declined further to 78.1 ± 13.7 pg/ml, significantly below that of nondiabetic controls (P < 0.01) (Figs. 2 and 3). The response to arginine alone and arginine plus glucose (Fig. 3) was also lower than at the onset of diabetes (NS). The mean age of this group was 136.8 ± 4.8 days.

Histologic Studies of the Endocrine Pancreas of BB/W Rats Before and at 14 Days After the Onset of Diabetes. In nondiabetic diabetes-prone BB/W rats endocrine tissue comprised 0.8 ± 0.2% of the pancreas at 60 days of age and 0.52 ± 0.22% at 140 days (NS; n = 4) (Fig. 4). In diabetes-resistant BB/W rats examined at 60 and 140 days of age the islets made up 0.78 ± 0.2 and 0.92 ± 0.56%, respectively. The value in 140-day-old nondiabetic diabetes-prone rats was significantly less than in the diabetes-resistant group at that age (P < 0.05; n = 4).

On the first day of the diabetes endocrine tissue made up 0.2 ± 0.1% of the pancreas (Fig. 4), and cells staining positively for insulin were reduced in number (Fig. 5c). On the 14th day of the diabetes, islet tissue made up only 0.07 ± 0.02% of the pancreas, and cells staining positively for insulin were extremely rare (Fig. 5d). Cells staining positively for glucagon and somatostatin were, however, observed.

Glucose Transport Rates in Islets Isolated from Rats 1 Day After the Onset of Diabetes. The glucose transport rate of islets isolated from rats 1 day after the beginning of the diabetes averaged 0.025 ± 0.008 nmol/min per islet, significantly less (P < 0.005) than the 0.419 ± 0.11 nmol/min per islet average in age-matched nondiabetic diabetes-prone littermates (Table 1). Because of the marked disparity in size between the islets

Fig. 3. Insulin and glucagon levels in venous effluent of perfused pancreata of 60-day-old nondiabetic diabetes-prone BB/W rats (Left) and diabetic BB/rats on the 1st (Center) and 14th days of the diabetes during perfusion with 10 mM of glucose, 10 mM arginine, and a combination of both (Right).

Fig. 4. Volume density of endocrine pancreas as percent of total pancreas in diabetes-prone (stippled bars) and diabetes-resistant (open bars) rats at 60 and 140 days of age and diabetic rats (solid bars) on the 1st and 14th days of diabetes. For all experiments, n = 4. *, P < 0.05 vs. preceding age; ○, not statistically significant.
of new onset diabetic rats, which were uniformly small (<90 μm in diameter), and those of age-matched nondiabetic diabetes-prone controls, which varied widely in size, the glucose transport rate per mg of protein and per μg of DNA was calculated. The differences between these groups remained strikingly significant (P < 0.005) (Table 1).

DISCUSSION

This study provides a chronicle of the pathophysiologic changes that occur in the endocrine pancreas of diabetes-prone BB/W rats before and immediately after the onset of overt diabetes. In pancreata of nondiabetic diabetes-prone BB/W rats, glucose-stimulated insulin secretion did not differ from that of age-matched diabetes-resistant controls at 60 days but was significantly less than the controls at 80 and 140 days of age. Arginine-stimulated insulin secretion, by contrast, did not differ at any age in the two groups. Baseline glucagon secretion in the nondiabetic diabetes-prone group also declined with age and at 140 days was significantly less than in diabetes-resistant littermates. The volume of the endocrine pancreas in nondiabetic diabetes-prone rats was the same as in diabetes-resistant controls at 60 days of age but at 140 days it was significantly below the controls.

These results suggest a progressive reduction in functional islet tissue in diabetes-prone rats prior to the onset of diabetes, in keeping with the insulitis that is present even in the absence of overt diabetes (14). They also suggest that this reduction occurs in diabetes-prone rats that probably would not have developed overt diabetes, since at 120 days only 15–20% of nondiabetic diabetes-prone rats would be expected to become diabetic (14). Current reliance on the appearance of overt diabetes as the criterion of phenotypic expression of this genetic autoimmune diathesis may result in underestimation of its penetrance.

On the first day of overt diabetes glucose-stimulated insulin secretion was completely absent, whereas arginine-stimulated insulin secretion was still quite normal. Baseline insulin secretion, which had declined progressively with age in both diabetes-prone and diabetes-resistant nondiabetic groups, was at the onset of diabetes about twice that of the 60-day-old nondiabetic diabetes-prone rats. But by the 14th day of the diabetes, baseline insulin levels were barely measurable (2.1 ± 0.1 microunits/ml), and both glucose and arginine-stimulated responses were absent, in contrast to reports (15, 16) of persistence of the insulin response to arginine well beyond this point.

Baseline glucagon secretion was significantly lower on the first day of diabetes but the response to arginine was somewhat increased; on the 14th day of the disease both baseline and arginine-stimulated glucagon secretion were slightly below normal, in confirmation of earlier studies (10, 15–17). These findings are consistent with the overall reduction in α cells (10) consequent to depletion of whole islets.

Table 1. Uptake of 3-O-methyl-D-glucose by islets of Langerhans from day 1 diabetic BB/W rats and age-matched nondiabetic diabetes-prone littermates

<table>
<thead>
<tr>
<th>3-O-methyl-D-glucose uptake</th>
<th>nmol/min per islet (n = 6)</th>
<th>nmol/min per mg of protein (n = 6)</th>
<th>nmol/min per μg of DNA (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prediabetic BB/W</td>
<td>0.419 ± 0.084</td>
<td>429 ± 76</td>
<td>14.4 ± 1.0</td>
</tr>
<tr>
<td>Day 1 diabetic BB/W</td>
<td>0.025 ± 0.003*</td>
<td>36 ± 6*</td>
<td>1.4 ± 0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*P < 0.005.
The near-normal glucagon secretory capacity (Fig. 3) of the reduced α-cell mass speaks for increased secretion by surviving α cells, perhaps as a consequence of a loss of normal insulin inhibition of glucagon secretion within the islets (18).

Pancreatic endocrine tissue was reduced by 75% relative to exocrine tissue on the first day of diabetes, and insulinstaining β cells were diminished compared to age-matched nondiabetic diabetes-prone controls. Although not analyzed morphometrically, this was probably the consequence of a decrease in both number and size of the islets. This is based on the impression that the islets isolated from new onset diabetic rats were far less numerous and smaller in maximum diameter (<90 μm) than in nondiabetic rats, in which islets range from 100 to 737 μm in diameter (19). DNA in islets isolated from six new onset diabetic rats averaged 25.96 ng per islet or about 3500 cells per islet. In islets from normal male albino Wistar and Sprague-Dawley rats, DNA ranges from 12 ng in the very smallest to 239 ng in the very largest, according to Parman (19). In islets of mixed size isolated from nondiabetic diabetes-prone BB/W rats, DNA levels average 56.6 ± 34.6 ng per islet (T.A., unpublished observations).

The age-related decline in glucose-stimulated insulin secretion in nondiabetic diabetes-prone rats and its absence at the onset of diabetes despite a normal response to arginine prompted studies of glucose transport in islets isolated on the first day of diabetes. When compared to islets from nondiabetic age-matched diabetes-prone rats, glucose transport rates were markedly reduced per islet (5% of controls), per mg of protein (8% of controls), and per ng of DNA (13% of controls). This was true even when the diabetic islets were compared with nondiabetic islets of similar size (<90 μm). This could, in part, reflect the relative increase in diabetic islets of non-β endocrine cells, which have slower transport rates for glucose than β cells (12), and in the infiltration by macrophages and lymphocytes.

Depletion of functioning β cells on the first day of diabetes was probably not sufficient to account for so large a defect in glucose transport, and other factors should be considered. Nonspecific functional damage to β cells consequent to ongoing autoimmune damage is a possible cause, although the normal insulin response to arginine argues for the functional integrity of at least some β cells. There is the possibility that there are two populations of β cells with different functions: a glucose-responsive population of islets or β cells within islets with a high rate of glucose transport that is preferentially destroyed early in the course of autoimmune destruction, and a glucose-unresponsive subpopulation with a low glucose transport rate that survives beyond the 1st day of overt diabetes but is destroyed by the 14th day. Finally, the fact that glucose-stimulated insulin secretion by the perfused rat pancreas is blocked by immunoglobulins from new onset type 1 diabetic subjects (20) raises the possibility of an antibody directed against some component of the glucose recognition-transport system.

The finding raises the possibility that the ubiquity of early loss of glucose-stimulated insulin secretion in etiologically unrelated forms of diabetes (1, 2, 21) could all reflect diverse alterations or depletion of a glucose-responsive subset of β cells.

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