Insulin release and pancreatic insulin is reduced in young prediabetic BB rats

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Abstract. The pancreases of approximately 50 days old diabetes-prone BB/Hagedorn (BB/H) and of the genetically closely related, but non-diabetic BB w-subline (control BB) rats were perfused to determine the capacity of D-glucose to release insulin before the expected development of diabetes. The BB/H rats were from a colony with 82–84% incidence of insulin-dependent diabetes mellitus (IDDM) by 140 days of age. The total amount of insulin released from the BB/H rat pancreas during stimulation with 20 mmol/l D-glucose was reduced by nearly 50% (P < 0.01). The initial peak of insulin release was similar between the two groups of animals, whereas the amount of insulin released during the second peak accounted for the diminished release (P < 0.01). The extractable pancreatic insulin was 30% (P < 0.05) less in the BB/H rats. Total insulin release expressed relative to the pancreatic insulin content, was therefore not different between the two groups. It is concluded that about 20–40 days before the mean age of clinical onset of IDDM in BB/H rats, the capacity to release insulin in response to D-glucose is reduced along with a diminished pancreatic insulin content. This abnormality seems to be preceded only by islet cell surface antibodies but not by insulitis.

The spontaneously insulin dependent diabetes mellitus (IDDM) in the BB rat which shows many homologies to IDDM in man, is clinically charac-

terized by an acute onset with weight loss, hyperglycaemia and glycosuria (Nakhooda et al. 1977). The pathogenesis of IDDM in the BB rat is not fully understood, and although the onset of diabetes is abrupt (Nakhooda et al. 1977), it is preceded by several immune abnormalities (Marliis et al. 1982). First, insulitis is detected about 10–16 days before onset (Logothetopoulos et al. 1984). Second, morphometric analyses indicate a decrease in endocrine and exocrine pancreas volumes already at 45 days of age (Klöppel et al. 1984). Third, islet cell surface and lymphocyte antibodies were found as early as at 38 days of age to have been present in some instances up to 70 days before onset (Dyrberg et al. 1984b; Pollard et al. 1983; Martin & Logothetopoulos 1984). Finally, islet cell antibodies detecting a Mr 64 000 rat islet autoantigen may be detected already at 33 days of age (Bækkeskov et al. 1984), only preceded by neonatal lymphopenia (Jackson et al. 1981; Poussier et al. 1982; Dyrberg et al. 1984a) although the latter abnormality does not seem to be a prerequisite for diabetes to develop (Like et al. 1985). Since these immune abnormalities preceded the appearance of insulitis, considered to be the cause of B-cell destruction, the aim of the present study was to test whether the B-cell function was altered in 50 days old BB/Hagedorn (BB/H) rats taken from a colony with a high incidence of IDDM.
Materials and Methods

Animals
The BB rats, kept in brother-sister breeding at the Hagedorn Research Laboratory colony originate from the Worcester colony of the University of Massachusetts (Butler et al. 1983). Two lines of BB rats, both inbred for 18–19 generations were studied. The diabetic line (BB/H) representing a continuation of the BB subline (Butler et al. 1983) from the 10th generation of inbreeding, showed an incidence of IDDM in the F2 and F5 generations of sister-brother breeding at our laboratory of 82% and 84%, respectively, with a mean age at onset of 89 days. The control BB rats represent the Worcester BB rat w-subline derived in the fifth generation from litter mates to the BB subline but producing no diabetes in subsequent generations of sister-brother breeding (Butler et al. 1983). The rats, kept in a light, temperature, and humidity controlled facility, were weaned at about 30 days of age and placed in cages three by three.

A total of 13 male BB/H rats from 8 different litters in the F2 and F5 generations and 10 male control BB rats from 5 different litters were studied (Table 1). The rats were kept overnight (18 h) without food but received 10% (w/v) sucrose in H2O ad libitum. This regimen was used for two reasons. First, it prevented hypoglycaemia induced by fasting and second ensured an empty stomach which was needed for technical reasons to carry out the operation before perfusion.

Blood glucose
Blood glucose concentration and body weight was determined before and after an overnight fast preceding the pancreas perfusion. Blood glucose in tail-vein blood samples, obtained from unanaesthetized rats was determined by a glucose-oxidase stix and reflectometer method.

Pancreas perfusion
The animals were anaesthetized by ip injections of sodium pentobarbital (50 mg/kg body weight). The pancreas perfusion was carried out as previously described for mice (Bonnevie-Nielsen et al. 1983; Svenningsen & Bonnevie-Nielsen 1984), with minor modifications to compensate for the larger size of the rats. The perfusion buffer kept at 37°C was pumped at a constant flow of 3 ml/min through the pancreas by a peristaltic pump without re-cycling. The perfusion pressure was approximately 40 mmHg during the entire perfusion. The perfusion apparatus was equipped with two medium chambers with perforated rollers to permit oxygenation and with a valve to allow a rapid shift between the two media. The perfusion buffer was a Krebs-Ringer-bicarbonate-Hepes buffer (KRBH), pH 7.4, with the following composition: NaCl 115 mmol/l, KCl 4.7 mmol/l, CaCl2 2.56 mmol/l, KH2PO4 1.2 mmol/l, MgSO4·7H2O 1.2 mmol/l, NaHCO3 24 mmol/l, Hepes 20 mmol/l, supplemented with 4% (w/v) bovine serum albumin, fraction V (Miles Laboratories Inc., Elkhart, IN). Before perfusion the buffer was passed through a Millipore filter (0.22 µm) and equilibrated with O2/CO2 (95/5%) for 45–60 min (90 l/min) at 40°C, to provide oxygen to the tissue and to maintain pH at 7.4.

The rat was placed in a warm chamber to keep the tissue at a constant temperature of 37°C during perfusion. The pancreas was perfused first for 20 min with KRBH buffer containing 3 mmol/l D-glucose. Thereafter, 3 min fractions were collected for a 12 min period to determine the basal release of insulin before the perfusion medium was changed to KRBH containing 20 mmol/l D-glucose. During the glucose-stimulation, fractions were first collected each minute for 8 min and then at 3 min intervals for up to 42 min. The fractions were collected on ice, aliquoted into separate tubes, frozen immediately and kept at -20°C for no longer than one month before determination of insulin.

Table 1.
Clinical data and organ weights of control BB and BB/Hagedorn rats. Body weight was determined after 18 h without food but with 10% (w/v) sucrose ad libitum. The blood glucose concentration in tail vein blood was determined by a stix-reflectometer method before perfusion. The two gonadal fat pads were removed after perfusion and the mean weight entered as one observation. The dry weight of the pancreas was determined after lyophilization. Median values and ranges within parentheses are shown.

<table>
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<tr>
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<th>Control BB rats</th>
<th>BB/Hagedorn rats</th>
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<tr>
<td>Males (n)</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Age (days)</td>
<td>46 (44–51)</td>
<td>51 (44–53)</td>
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<tr>
<td>Body weight after fastig (g)</td>
<td>151 (117–187)</td>
<td>132 (83–168)</td>
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<tr>
<td>Decrease in body weight after fasting (%)</td>
<td>6.6 (3.9–9.8)</td>
<td>6.1 (4.4–10.7)</td>
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<tr>
<td>Blood glucose concentration after fasting (mmol/l)</td>
<td>4.7 (4.0–5.3)</td>
<td>4.2 (3.4–5.1)</td>
</tr>
<tr>
<td>Gonadal fat pad wet weight (mg)</td>
<td>330.2 (131.5–369.8)</td>
<td>255.9 (89.4–452.6)</td>
</tr>
<tr>
<td>Pancreas dry weight (mg)</td>
<td>212.5 (126.3–248.5)</td>
<td>165.7 (89.9–307.3)</td>
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After perfusion the pancreas was excised, snap-frozen in liquid nitrogen and stored for max 3 months at 
-80°C before insulin extraction. The gonadal fat pads were removed and weighed, and the mean value of the 
two used to estimate the total body fat (Svenningsen & Bonnevie-Nielsen 1984).

Radioimmunoassay of rat insulin

Insulin was determined in a radioimmunoassay using a 
guinea-pig anti-insulin serum (GP12) as the first anti-
body (final dilution was 1:10⁵ [¹²⁵I] [¹⁴C]iodoalbumin (Nordisk Gentofte A/S, Gentofte, Denmark) as 
tracer, and rabbit anti-guinea-pig immunoglobulin as 
second antibody (Dakopatts, Copenhagen, Denmark). 
Rat insulin was used as standard (Batch 410, Novo A/S, 
Denmark). Non-specific binding was less than 0.5%, and 
the detection limit was 0.015 pmol/ml. Serial dilu-
tion showed linearity between 0.09 and 0.83 pmol/ml. 
The coefficient of variation of independent replicates 
was 6.4% (n = 14).

Pancreatic insulin extraction

Each pancreas was freeze-dried, weighed and cut into 
small pieces and extracted by shaking (200 r.p.m) at 4°C 
for 24 h in 20 ml phosphoric acid ethanol composed of 
0.33% (v/v) concentrated H₃PO₄ in 70% ethanol (pH 
2.7). The extract was centrifuged (4°C, 10 min at 10000 
× g) and an aliquot of the supernatant diluted in 
KRBH buffer before insulin analysis. More than 95% of 
the total extractable pancreatic insulin was extracted 
after 24 h and the recovery of [¹²⁵I]insulin added 
during extraction exceeded 95%.

Statistical evaluation

The Mann-Whitney U-test was used to test differences 
between experimental and control animals.

Results

The BB/H and the control BB rats did not differ in 
body weight, the relative reduction in body weight and 
the blood glucose concentration following 18 h fasting with 10% sucrose (Table 1). The median values of the BB/H rat body weight, 
gonadal fat-pad weight and whole pancreas dry weight were lower, but did not differ from the 
control BB rats (Table 1). An extended analysis of 
the growth pattern demonstrates that male control 
BB rats and BB/H rats show comparable body weights during the first four weeks of life, but that 
the BB/H rats thereafter tend to lag behind the 
control rats.

The dynamics of insulin release following glu-
cose stimulation was comparable in the two 
groups of rats showing a rapid increase in insulin 
release immediately after glucose stimulation, 
followed by a nadir and a more slowly increasing 
second phase (Fig. 1). However, in the BB/H rats 
the second phase of insulin release was less pro-
nounced (Fig. 1), in fact, the BB/H rats released 
significantly lower amounts of insulin during the 
second phase (Table 2).

The pancreatic insulin content after perfusion 
in the BB/H rats was also significantly lower than 
in the controls BB rats (Table 2). The relative 
pancreatic insulin content i.e. insulin per pancreas 
dry weight in BB/H and control BB rats was not 
statistically different. The total amount of insulin
released during glucose stimulation, expressed relative to the pancreatic insulin content, showed no difference between two groups.

**Discussion**

In this report it is shown that about 50 days old BB/H rats, i.e. about 20–40 days before the expected onset of IDDM, have functional reduction in the insulin response to glucose stimulation compared to matched non-diabetic control BB (w-subline) rats. The pancreas perfusion technique has previously been used to demonstrate a profound decrease in insulin release in overtly diabetic BB rats at or after the onset of IDDM (Poussier et al. 1983; Grill & Herberg 1983; Boden et al. 1983; Ruggere & Patel 1984). In order to study BB rats which are claimed to be pre-diabetic, it is necessary that the incidence of IDDM in the colony is high and persistent throughout several generations of inbreeding. In our colony, the incidence of IDDM has remained at 82–90% during the last 7 generations of sister-brother breedings, and has shown an even distribution among the litters within each generation. Among the 13 BB/H rats studied, we would therefore have expected 11 rats to develop IDDM. Although diabetes was recently reported in the w-subline of the Worcester colony (Like et al. 1985) this has not been seen among our control BB rats.

The low glucose-stimulated insulin release in our BB/H rats is unlikely to be explained by the larger body weight of the control BB rats since the total insulin release relative to body weight was still significantly different (median values of BB/H vs control BB rats: 1.81 vs 2.86 pmol insulin/g body weight, P < 0.02). The total amount of extractable pancreatic insulin was significantly lower in the BB/H rats. There was, however, no difference when the relative pancreatic insulin content was compared. These results suggest that the mechanisms by which glucose stimulates insulin release are not impaired and that the decrease in insulin release primarily reflect a lower pancreatic insulin content in matched BB/H and control rats.

The pathogenesis of IDDM in BB rats appears to include an autoimmune destruction of the pancreatic B-cells (Marliss et al. 1982; Dyrberg et al. 1984b). We speculate that the autoimmune activity directed against the B-cells occurs a considerable time before the clinical onset of IDDM and results in a reduced insulin release capacity.

### Table 2.
Amount of insulin released during glucose stimulation and pancreatic insulin content after perfusion of approximately 50 days old BB/Hagedorn and control BB rats. BB/Hagedorn and control BB rats, approximately 50 days of age, were subjected to perfusion with buffer containing 3 mmol/l of D-glucose, and the buffer was changed to 20 mmol/l at 13 min. The total amount of insulin released during the first peak is shown in a), the second peak in b) and total insulin release during the glucose stimulation in c). Total pancreatic insulin is total insulin released added to the residual insulin content. The groups were compared by the Mann-Whitney test. N.S. is not significant. Median values and ranges are shown.

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<th>Control BB rats</th>
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<tr>
<td><strong>Total insulin release (pmol)</strong></td>
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<tr>
<td>a) 13–19 min</td>
<td>20.62 (8.99–59.29)</td>
<td>18.48 (7.74–49.38)</td>
<td>N.S.</td>
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<tr>
<td>b) 20–62 min</td>
<td>401.38 (227.23–528.55)</td>
<td>202.48 (141.83–356.42)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>c) 13–62 min</td>
<td>443.20 (236.20–573.93)</td>
<td>217.27 (153.55–380.03)</td>
<td>&lt; 0.01</td>
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<tr>
<td><strong>Residual pancreatic insulin content (nmol)</strong></td>
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<td></td>
<td>12.38 (6.35–17.47)</td>
<td>8.73 (3.27–15.66)</td>
<td>&lt; 0.05</td>
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<tr>
<td><strong>Total pancreatic insulin content (nmol)</strong></td>
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<td></td>
<td>13.05 (6.8–17.80)</td>
<td>9.15 (3.46–15.83)</td>
<td>&lt; 0.05</td>
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<td><strong>Pancreatic insulin (pmol)/pancreas dry weight (mg)</strong></td>
<td>67.3</td>
<td>42.7</td>
<td>N.S.</td>
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<td>(40.6–82.2)</td>
<td>(24.9–98.2)</td>
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<tr>
<td><strong>Total insulin release/pancreatic insulin content</strong></td>
<td>4.00</td>
<td>2.64</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>(1.50–7.04)</td>
<td>(1.08–6.31)</td>
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and pancreatic insulin content. It is possible that adverse immunological reactions towards the pancreatic B-cells, most prominently reflected by the presence of autoantibodies against the M_{6} 64 000 antigen already in 20 days old BB/H rats (Bækkeskov & Lernmark 1985), may cause a loss of B-cells, not sufficient to cause hyperglycaemia and altered glucose metabolism, but sufficient to preclude a normal growth of the endocrine and exocrine pancreas. An escalating immune activity against the B-cells would later, 10–14 days before onset (Logothetopoulos et al. 1984) mobilize inflammatory cells to the islets of Langerhans to cause the B-cell destruction necessary to precipitate IDDM.

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References

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