Glucose- and arginine-induced insulin and glucagon responses from the isolated perfused pancreas of the BB-Wistar diabetic rat. Evidence for selective impairment of glucose regulation

V. Grill and L. Herberg

Department of Endocrinology, Karolinska Hospital, S-104 01 Stockholm and Diabetes Forschungsinstutit an der Universität Düsseldorf, Düsseldorf, FRG

Abstract. To investigate whether preferential responsiveness of residual B-cells is a feature of a diabetic state we compared insulin-releasing effects of glucose and arginine in perfused pancreases from moderately diabetic BB-Wistar rats. BB-rats were hyperglycaemic and insulin-dependent but possessed some insulin reserves (6 per cent of pancreatic content of control Wistar rats). Glucose (27.7 mM) failed to release insulin from diabetic pancreases while, conversely, arginine (8 mM) evoked a several-fold increase in insulin secretion. Ratios between responses from diabetic and normal pancreases were 0.01 and 0.29, respectively, when glucose or arginine were used as stimuli. This difference was significant ($P < 0.05$, Wilcoxon test). Glucose furthermore failed to exert a time-dependent (= priming) effect on arginine-induced insulin secretion in the diabetic animals. Also A-cell responsiveness to glucose (acute and priming effects) were lost in BB-rats. It is concluded that selective loss of glucose effects on B- and A-cell secretion are associated with the diabetic state of the BB-Wistar rats.

Evidence indicates that in type 2 (non-insulin-dependent) diabetes, non-glucose stimuli may release insulin also when glucose is ineffective (Deckert et al. 1972; Robertson & Porte 1973; Palmer et al. 1976). While these findings may suggest an inherent secretory defect in the recognition of glucose as secretagogue it is also possible that insensitivity to glucose could be secondary to conditions induced by the diabetic state such as hyperglycaemia, other metabolic abnormalities or a state of hyperactivity of the B-cell. One way to study the importance of inherent vs exogenous factors would be to investigate preferential effects of secretagogues in type 1 diabetes where a diabetic state would appear to be unequivocally secondary to wide-spread destruction of B-cells rather than to any inherent defect in these cells.

The Wistar BB-rat fulfils many criteria for an animal model of human type 1 diabetes (Nakhooda et al. 1977; Marliss et al. 1982). As in human type 1 diabetes, the degree of severity of the diabetic state of the BB-rat varies in association with a higher or lesser degree of B-cell destruction. We used moderately diabetic BB-Wistar rats to test whether or not stimulation of insulin secretion by ambient glucose was preferentially lost in comparison with response to arginine in the perfused pancreas preparation. Additionally, since glucagon secretion is normally influenced both by glucose and by arginine (for review see Gerich et al. 1976) we also looked for an influence of the diabetic state on glucagon responses. Experiments were designed to compare in the diabetic and non-diabetic state not only the acute but also the time-dependent (= priming) secretory effects of glucose which have previously been demonstrated (Grodsny et al. 1969; Cerasi 1975; Grill et al. 1978, 1979).
Materials and Methods

Animals

Diabetic and non-diabetic control rats were derived from an outbred colony of Wistar rats maintained at the Bio Breeding Laboratories in Ottawa, Canada. In 1979 weanlings were shipped to the Diabetes Forschungsinstitut in Düsseldorf. Subsequent breeding scheme followed strict brother × sister mating. All rats used were male F3. Although there is not doubt about the genetic transmission of the diabetic state the precise nature is still obscure. Therefore we cannot exclude in our controls the presence of a genetic trait for diabetes which was phenotypically not detectable.

Diabetes was diagnosed from the appearance of glycosuria between day 117 and 418 of age. From that day on the animals were treated with one daily injection of intermediate acting porc insulin (Insulin Novo Lente 0.2–2.0 U/100 g body weight sc). Insulin doses were adjusted to blood glucose levels, determined once a week, and to body weight, which was daily determined. Blood glucose was determined enzymatically. Animals were air-freighted to Stockholm where they were allowed to adapt for 14 days, or until their weights were stable. The animals always had free access to unacidified water and a commercial pelleted food.

Experimental procedure

The animals received their last insulin injection at 3 p.m. on the day preceding the experiment when the pancreas was isolated and perfused as described (Loubatières et al. 1969; Grill et al. 1978). The pancreas was perfused with Krebs-Ringer-bicarbonate medium (KRB), containing 2 g/100 ml bovine albumin (Sigma, St Louis, Missouri) and – when not otherwise indicated – 3.9 mM/l of D-glucose (= ‘basal’ medium). Flow rates were between 4 and 5 ml/min throughout the experiments. The pancreas was perfused with ‘basal’ medium during a 20 min equilibration period (not indicated in Figs.). Perfusate was sampled in tubes containing each 1000 IU aprotinin (Trasylo1) per ml, frozen and later assayed for insulin (Herbert et al. 1965) and glucagon (Faloona & Unger 1974). At the end of experiments the perfused pancreas was frozen and later extracted and assayed for insulin and glucagon (Grill & Efendić 1983).

Table 1.

Characteristics of diabetic Wistar BB rats and their controls.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Diabetes</th>
<th>Blood glucose (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Onset</td>
<td>Duration</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(days of age)</td>
<td>(days)</td>
</tr>
<tr>
<td>7/10 oh</td>
<td>366</td>
<td>460</td>
<td>175</td>
<td>191</td>
</tr>
<tr>
<td>12/2 re</td>
<td>417</td>
<td>404</td>
<td>373</td>
<td>44</td>
</tr>
<tr>
<td>12/4 h rel</td>
<td>463</td>
<td>512</td>
<td>418</td>
<td>45</td>
</tr>
<tr>
<td>12/5 a li</td>
<td>295</td>
<td>455</td>
<td>135</td>
<td>160</td>
</tr>
<tr>
<td>12/10 re</td>
<td>244</td>
<td>426</td>
<td>166</td>
<td>78</td>
</tr>
<tr>
<td>2/25 re</td>
<td>187</td>
<td>466</td>
<td>117</td>
<td>70</td>
</tr>
<tr>
<td>11/5 oh</td>
<td>550</td>
<td>573</td>
<td>468</td>
<td>82</td>
</tr>
<tr>
<td>12/4 li</td>
<td>446</td>
<td>407</td>
<td>151</td>
<td>295</td>
</tr>
<tr>
<td>12/9 d re</td>
<td>505</td>
<td>489</td>
<td>174</td>
<td>331</td>
</tr>
</tbody>
</table>

(\bar{x} ± SEM) 385 ± 41 465 ± 18 241 ± 45 144 ± 36 14.0 ± 1.2 24.6 ± 2.4

| (n = 10) Controls | 365 ± 34 | 539 ± 19 | – | – | – |

* Numbers of blood glucose determinations.
Effects of arginine (8 mM) or glucose (27.7 mM) on insulin secretion. Mean ± SEM of the number of experiments indicated in Table 2.

### Results

**Characteristics of BB-Wistar rats**

Diabetic and control rats were of similar age (Table 1); the mean body weight was however higher ($P < 0.02$) in controls. Blood glucose concentration of diabetic rats before being shipped to Stockholm was $14.0 \pm 1.2$ mM. Blood glucose values from a single determination in 6 control animals were $6.2 \pm 0.2$ mM. Blood glucose was elevated immediately after the pancreatic isolation both in the diabetic and control rats (Table 1); the levels were however highest in the diabetics. (Elevated concentrations of glucose in the controls were presumably due to the surgical stress).

Pancreatic insulin content in diabetic rats was 6% of that of the controls ($292 \pm 96$ vs $5168 \pm 545$ mU/pancreas) ($P < 0.001$ for difference). Glucagon content was not significantly different between groups ($4.5 \pm 0.6$ µg per diabetic vs $3.2 \pm 0.8$ µg per control pancreas).

**Insulin secretion in vitro**

*Normal rats.* Both glucose (27.7 mM) and arginine (8 mM) stimulated insulin secretion from pancreases of normal rats (Fig. 1). When an initial infusion of arginine (arginine 1) was followed by a second pulse of arginine (arginine 2) (lower part of Fig. 1) the insulin response to arginine 2 was clearly inhibited relative to arginine 1 (Fig. 1, Table 2). When a 20 min glucose infusion preceded arginine 2 (upper part of Fig. 1) the response to arginine 2 was however greater than to arginine 1 and 6-fold increased relative to arginine 2 in the control situation (Table 2, compare first and second lines). Hence it was apparent that previous glucose exerted not only an acute but also a priming effect on arginine-induced insulin secretion in normal rats.
**Diabetic rats.** Insulin secretory responses were diminished in pancreases from the diabetic rats (Fig. 1, Table 2). However, whereas the response to glucose was almost or totally abolished, a moderate response to arginine could still be evoked. A selective loss of the glucose response in the BB-rats was apparent when the responses to arginine and glucose were compared in the diabetic and normal rats. The ratio of the response to glucose and arginine between the two groups was 0.01 and 0.29, respectively. This difference between the ratios was significant ($P < 0.05$, Wilcoxon test).

In contrast to the results with normal pancreases, the insulin response to arginine 2 was not diminished relative to that evoked by arginine 1 when the priming infusion of glucose was omitted. Prior exposure to glucose did not change arginine-induced release. Hence a priming effect by glucose was absent in diabetic pancreases.

**Glucagon release**

An initial infusion of arginine evoked similar glucagon responses from normal and diabetic pancreases (Fig. 2.) Conversely, while, during its presence, 27.7 mM/l of glucose inhibited (albeit tran-
siently, between min 20–25 and 25–35, $P < 0.05$) "basal" glucagon release from normal pancreases, no such inhibition by glucose was evident in the diabetic pancreases (Fig. 2, Table 2). As to a priming effect by glucose, this was not evident from the comparison between responses to arginine 1 and 2 which were similar both in normal and diabetic pancreases despite an intervening infusion of glucose (upper part of Fig. 2, Table 2). However in normal pancreases, a priming effect by glucose was suggested from experiments shown in the lower part of Fig. 2, which indicate that the response to arginine 2 was actually enhanced in the absence of a preceding infusion of 27.7 mM/l of glucose. In contrast, no such enhancement and consequently no indication of a priming effect was obvious in the diabetic pancreases.

### Discussion

The main finding of the present study is the preferential loss of insulin release in response to glucose in the BB-rats. Weir et al. (1981) have demonstrated a similar defect of the insulin re-

### Table 2.

Effects of arginine and glucose on insulin and glucagon secretion. Results are expressed as integrated area of secretion, above basal secretory rates (= secretion rates immediately preceding the introduction of secretagogues). Significance testing 1:III vs 2:III, $P < 0.01$, 1:VI vs 2:VI, $P < 0.05$, 2:1 vs 2:III, $P < 0.01$.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>No. of experiments</th>
<th>Insulin (μU)</th>
<th>Glucagon (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I Arginine</td>
<td>II Glucose (27.7 mM/l)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>min 0–5</td>
<td>min 15–35</td>
</tr>
<tr>
<td>Control rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Protocol A (arginine-glucose-arginine)</td>
<td>5</td>
<td>13 044 ± 4 083</td>
<td>15 576 ± 6 356</td>
</tr>
<tr>
<td>2. Protocol B (arginine-arginine)</td>
<td>5</td>
<td>12 632 ± 4 225</td>
<td>–</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Protocol A (arginine-glucose-arginine)</td>
<td>5</td>
<td>4229 ± 1 887</td>
<td>356 ± 2 40</td>
</tr>
<tr>
<td>4. Protocol B (arginine-arginine)</td>
<td>4</td>
<td>3934 ± 899</td>
<td>–</td>
</tr>
</tbody>
</table>

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Effects of arginine (8 mM) or glucose (27.7 mM) on glucagon secretion. Mean ± SEM of the number of experiments indicated in Table 2.

Figure 2.

Response to glucose (and tolbutamide) as compared to arginine and isoproterenol in rats where diabetes had been induced by streptozotocin. In the latter model of diabetes it cannot be ruled out that the B-cell cytotoxic drug may have selectively damaged a putative glucoreceptor of the B-cell. Our results would seem to offer stronger evidence that a preferential lack of insulin response in animal and, by analogy, also in human diabetes can occur as a consequence of the diabetic state. Thus, to our knowledge, no evidence exists that B-cell damage in BB-rats specifically involves the handling of glucose in the islets.

Our results do not elucidate which environmental factor(s) that are responsible for the unresponsiveness of the diabetic B-cell. In search of an explanation, it is interesting to note the loss of responsiveness to glucose in diabetes both with regard to A cell (review Gerich et al. 1976, illustrated also by present results) and the D cell (review Hermansen 1980). It seems possible that factors coupled to insulinopenia and/or hyperglycaemia can affect the glucose responsiveness also of the B-cell. In this context it should be pointed out that the insulin treatment of our BB-rats was too weak to normalize hyperglycaemia and weight gain in these animals.

Arginine per se inhibited insulin secretion in control animals as judged by the observation that a second infusion of the amino acid – when administered without a preceding infusion of elevated glucose – elicited a much smaller insulin response than a first infusion of arginine in pancreas from normal rats. A similar effect of arginine has been documented also in man (Efendić et al. 1979). No explanation is presently available for this pheno-
menon, nor can we elucidate the reasons for the clear differences observed vis-à-vis the diabetic pancreas in which arginine seemed to have no inhibitory influence. It is possible that arginine, which itself is not metabolized in islets (Hellman et al. 1971) may adversely interfere with the metabolism of nutrients in the normal but not in the diabetic B-cell. If so, the present findings offer an indication that a diabetic state may importantly influence metabolic processes in the B-cell.

A priming effect of glucose on arginine-induced insulin as well as on glucagon secretion was abolished in the BB-rats. In alloxan-diabetic rats with almost no B-cell function insulin treatment was able to restore a priming effect on glucagon secretion (Grill & Efendic 1983). Hence acute and priming effects alike can be directly or indirectly affected by insulinopenia. Although acute and priming effects of glucose may be induced by partly different mechanisms (Grill et al. 1978) their dual loss in diabetes and their restoration by insulin treatment suggests an intimate relationship between different secretory effects of glucose on B-as well as on A-cells.

Acknowledgments

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References


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