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Absence of glucopenic inhibition of the insulin response to arginine at the onset of diabetes in BB/W rats

I. Komiya and R. H. Unger

Center for Diabetes Research, University of Texas Health Science Center, the Veterans Administration Medical Center and the Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas, USA

Summary. To determine if the inhibiting effect of glucopenia on arginine-stimulated insulin secretion is impaired at the onset of autoimmune diabetes, the insulin response to arginine was studied at 5.6 and 2.8 mmol/l glucose in perfused pancreata isolated from BB/W rats on the first day of diabetes and from age-matched diabetes-prone BB/W rats without diabetes. During glucopenia the baseline insulin secretion was reduced by more than 80% in both groups. However, the arginine-stimulated insulin response in the diabetic group was only 16.5% lower during glucopenia compared to 79.1% lower in the nondiabetic controls. Also, enhancement of the arginine-stimulated glucagon response by glucopenia was modest compared to controls. The results indicated that at the onset of this form of autoimmune diabetes the surviving B cells are, for unknown reasons, hyperresponsive to arginine and that, in contrast to the controls, this response is not inhibited by glucopenia.

Key words: Glucopenia, insulin, glucagon, arginine, diabetes, BB/W rats, perfused pancreas.

Under normal circumstances hyperglycaemia potentiates arginine-stimulated insulin secretion and glucopenia inhibits it [1-3]. At the onset of autoimmune diabetes in BB/W rats the potentiating effect of glucose on arginine-stimulated insulin secretion is absent [4], but it is not known if the inhibitory effect of glucopenia is also impaired. The present study was designed to determine the ability of glucopenia to inhibit arginine-stimulated insulin secretion at the onset of spontaneous autoimmune diabetes in BB/W rats.

Materials and methods

Male BB/W diabetes-prone rats were obtained from the University of Massachusetts at 50 days of age and fed ad libitum under controlled lighting conditions (12 h light alternating with 12 h darkness). Blood glucose levels were determined daily from tail blood samples using a Beckman Glucose Analyzer II (Beckman Co., Brea, Calif, USA). Urinary glucose was measured daily by using Ketodiastix. Five rats that became diabetic were studied on the first day of diabetes (defined as a fasting blood glucose level >11 mmol/l accompanied by glycosuria). On the day of the experiment the mean glucose level of the 5 rats was 13.6 ± 0.6 mmol/l and the mean age was 88 ± 3 days. Five non-diabetic diabetes-prone BB/W rats were used as controls; their mean glucose level on the day of the experiment was 6.9 ± 0.2 mmol/l and their mean age was 83 ± 3 days, which did not differ statistically from the diabetic group.

On the day of study rats were anaesthetised with 50 mg/kg sodium pentobarbital injected intraperitoneally. The pancreata were isolated and perfused by the method of Grodsky and Fanska [5], as previously described in detail [6]. The perfusate was a Krebs-Ringer bicarbonate buffer containing 5 mmol/l of pyruvate, fumarate, glutamate and 5.6 mmol/l glucose. The flow rate was 2.7 ml/min. Following a 10-min equilibration period the effluent was collected in chilled tubes at 1-min intervals. After a 10-min baseline perfusion period, 10 mmol/l arginine hydrochloride was perfused for 10 min. Five minutes later the glucose concentration was lowered to 2.8 mmol/l and after a 10-min baseline interval 10 mmol/l arginine was perfused again for 10 min.

Insulin was measured by the method of Yalow and Berson [7] with modification [8]. Glucagon was measured by a previously described method using 30K [9]. The integrated hormone response to given stimulus was the total of the 10 incremental values during 10-min of stimulation.

Statistical analysis

All data were expressed as means $\pm$ SEM. The statistical significance of difference was estimated by paired and non-paired Student's t-test.

Results

Effect of glucopenia on baseline and arginine-stimulated insulin secretion by pancreata of diabetic and nondiabetic BB/W rats

Baseline insulin secretion in both BB/W diabetic and nondiabetic rats was much lower at 2.8 mmol/l glucose than at the 5.6 mmol/l concentration ($p<0.001$ and $p<0.05$ respectively) (Fig. 1); the reduction in the 2 groups averaged $83.3 \pm 1.8\%$ and $88.6 \pm 3.6\%$ respectively. However, in the diabetic group the insulin response to 10 mmol/l arginine was not fully inhibited by glucopenia; in nondiabetic rats the insulin response to arginine at 2.8 mmol/l glucose was $20.9 \pm 4.3\%$ of

that at 5.6 mmol/l glucose ($p < 0.01$), whereas in diabetic rats it was 83.5 ± 8.9% of the response to 5.6 mmol/l glucose (NS). The insulin response to arginine during glucopenia was 2-fold greater in diabetic rats than in normal rats (1.52 ± 0.22 mU/10 min vs 0.69 ± 0.20 mU/10 min; $p < 0.05$) (Figs. 1 and 2).

Baseline and arginine-stimulated glucagon secretion at 5.6 mmol/l or 2.8 mmol/l glucose in diabetic and nondiabetic BB/W rats

In nondiabetic rats the baseline glucagon level was not altered by the lower glucose concentration (Fig. 3). In diabetic rats it was paradoxically reduced, averaging 67.1 ± 6.9 percent less at 2.8 mmol/l glucose than at the 5.6 mmol/l concentration ($p < 0.01$). In the diabetic rats the glucagon response to 10 mmol/l arginine at 5.6 mmol/l glucose was almost twice that of the nondiabetic rats (23.10 ± 2.63 ng/10 min vs 12.43 ± 1.70 ng/10 min; $p < 0.01$) (Figs. 2 and 3), but the enhancement by glucopenia was modest (23.10 ± 2.63 ng/10 min vs 30.53 ± 3.94 ng/10 min; $p < 0.05$), compared to the two-fold enhancement in the nondiabetic rats (12.43 ± 1.70 ng/10 min vs 32.24 ± 3.85 ng/10 min; $p < 0.01$).

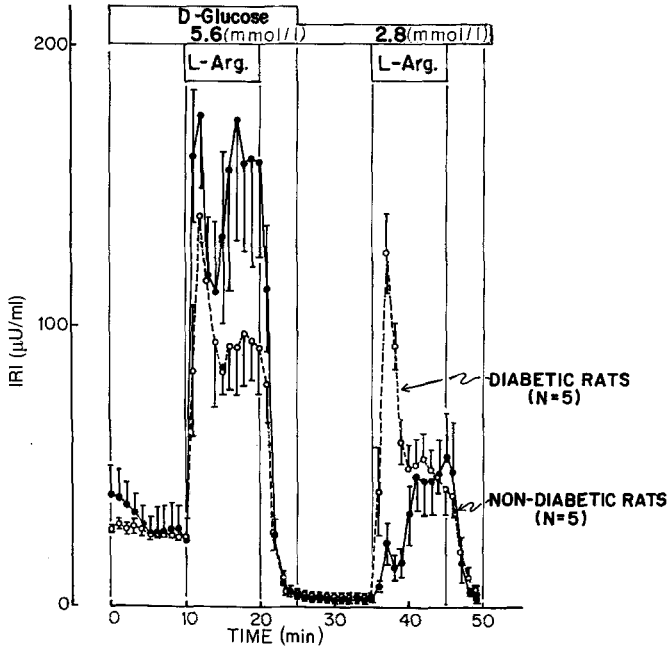

Fig. 1. The mean (±SEM) response to arginine at normal and low glucose concentrations in the perfused pancreas of new onset diabetic BB/W rats and age-matched nondiabetic BB/W control rats

Discussion

This study examines the effect of glucopenia upon baseline and arginine-induced insulin and glucagon secretion in pancreata isolated from newly diabetic BB/W rats and from nondiabetic BB/W control rats. The baseline secretion of insulin was markedly reduced by glucopenia in both groups. In the diabetic group the insulin response to arginine at the 5.6 mmol/l glucose concentration was as great as in the controls, thus confirming earlier work [4]. However, in the diabetic group it was only 20% lower during glucopenia, compared to 80% lower in the nondiabetic group. In fact, at the 2.8 mmol/l glucose concentration the arginine-induced insulin response in diabetic BB/W rats was more than twice that of nondiabetic BB/W controls ($p < 0.05$), despite the fact that only 20% of the normal complement of B cells are still present on the first day of diabetes [4]. Thus, both the enhancing effect of a high glucose concentration and the inhibitory effect of a low glucose concentration upon arginine-induced insulin secretion are absent at the onset of diabetes in BB/W rats. The latter abnormality has been observed in diabetic patients [10] and in animal models of streptozotocin-induced diabetes [11], but has not been reported in spontaneous autoimmune diabetes.

The mechanism of these abnormalities is unclear. One possibility is that the ability to recognise transport and/or metabolise glucose is somehow altered in the B cells that are still present and functioning on the first day of BB/W diabetes [12]; indeed, the glucose transport activity in the islets of BB/W rats on the first day

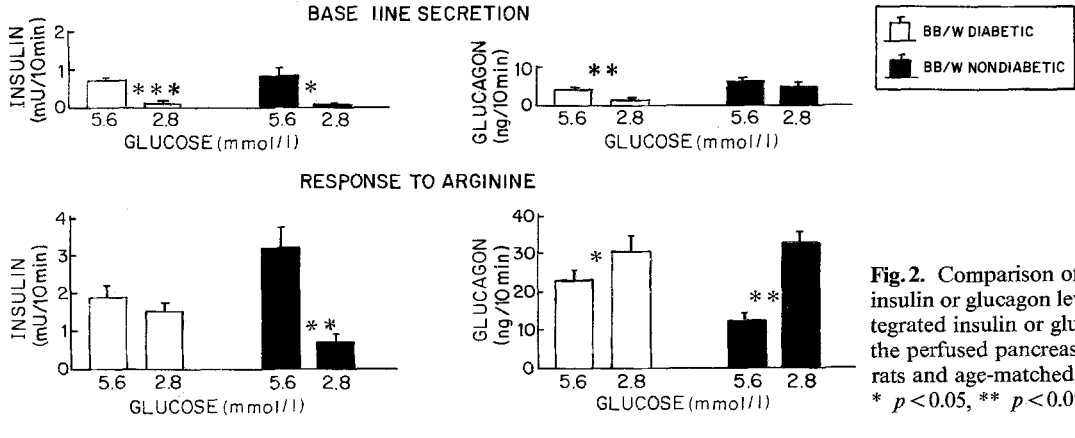

Fig. 2. Comparison of the mean (±SEM) baseline insulin or glucagon levels and the mean (±SEM) integrated insulin or glucagon response to arginine in the perfused pancreas of new onset diabetic BB/W rats and age-matched nondiabetic control rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

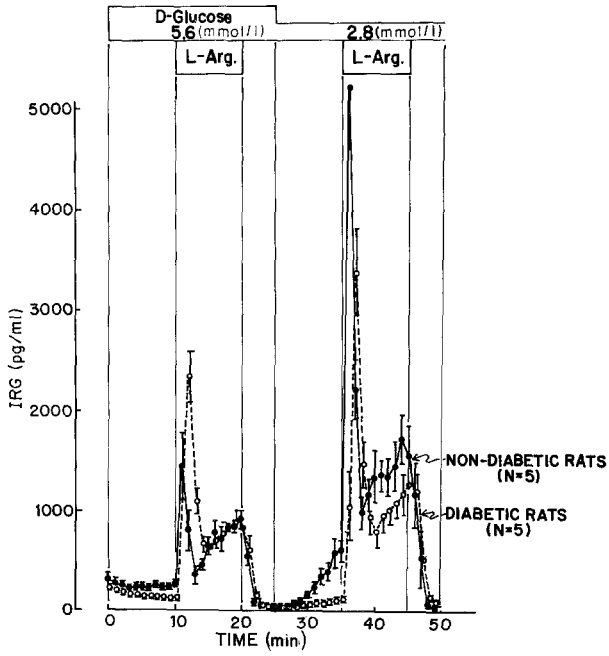

Fig. 3. The mean (±SEM) glucagon response to arginine at normal and low glucose concentration in the perfused pancreas of new onset diabetic BB/W and age-matched nondiabetic BB/W control rats

of their diabetes has previously been reported to be markedly reduced [4]. Hyperglycaemia itself has been invoked as a reversible cause of B-cell insensitivity to glucose [13]. Yet in these studies baseline insulin secretion of diabetic pancreata was inhibited by glucopenia to the same degree as in normal ones (Fig. 2). A second possibility suggested in studies of non-insulin-dependent diabetes in adult rats following neonatal streptozotocin is that the B cells are hypersensitive to arginine [14]. A third possibility is that glucopenic inhibition of B cells is mediated by nor-epinephrine released from local adrenergic nerve-endings [6] and that these anatomical relationships are disrupted by insulitis and B-cell destruction [15]. However, it has been reported that arginine-induced insulin secretion is not blocked by epinephrine [16].

The normal effect of glucopenia to enhance arginine-induced glucagon secretion [13] was also absent in the newly diabetic rats. Perhaps at the 5.6 mmol/l glucose concentration arginine-stimulated glucagon secretion was already near maximal levels, and incapable of a further increase. Alternatively, a generalised loss of glucose recognition by islet cells has been proposed to explain the impairment of certain islet cell responses to glucose in diabetes [17].

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Dr. R. H. Unger
Center for Diabetes Research
University of Texas
Southwestern Medical School
5323 Harry Hines Boulevard
Dallas, Texas 75235-9030
USA