B-cell responses to intravenous glucose and glucagon in non-diabetic twins of patients with Type 1 (insulin-dependent) diabetes mellitus

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Summary. The B-cells of patients with recently diagnosed Type 1 (insulin-dependent) diabetes may have no response to glucose when the response to glucagon is present but attenuated. This observation suggests that the recognition of glucose is more severely affected than that for non-glucose stimulants. To determine whether a similar selective decrease in glucose response was present before the onset of diabetes we studied two groups of non-diabetic identical twins of patients with recently diagnosed Type 1 diabetes: one group with complement-fixing islet cell antibodies who were at high risk of developing diabetes (four of the five have already developed diabetes) and a group without such antibodies at low risk of developing diabetes. In addition, a group of patients with chronic pancreatitis were studied to control for non-specific damage to the B-cell. Responses to i.v. glucose and i.v. glucagon were compared.

Patients with chronic pancreatitis has similar responses to both glucose and glucagon and the responses did not differ from control subjects. The B-cells of the immune positive group showed evidence of pathology because the insulin and C-peptide responses to both stimuli were reduced when compared to either their control subjects or the immune negative twin group. However, the B-cell response to both glucose and glucagon in the immune positive twins was similar. Because the B-cell response to glucose was not less than that to glucagon, a selective destruction of the glucose recognition system cannot be a characteristic of all twins throughout the period before they develop Type 1 diabetes.

Key words: B-cell responses, i.v. glucose, i.v. glucagon, non-diabetic twins.

Type 1 (insulin-dependent) diabetes is due to specific destruction of insulin secreting B-cells of the pancreas. The disease process leading to Type 1 diabetes is slow, occurring over many months and associated with a gradual deterioration in glucose tolerance and a decline in the insulin response to i.v. glucose [1, 2]. Three recently diagnosed patients with Type 1 diabetes have been reported as having no B-cell response to glucose but an attenuated response to glucagon [3]. It is not known at which stage of the disease the B-cells first show this pattern of response though it has been described in three non-diabetic relatives of Type 1 diabetic patients [2]. In order to determine whether the B-cells of non-diabetic individuals genetically susceptible to Type 1 diabetes could show a decreased response to glucose compared to glucagon throughout the period before they develop diabetes, we studied two groups of non-diabetic identical twins of Type 1 diabetic patients; one group with complement-fixing islet cell antibodies who were at high risk of developing Type 1 diabetes and another group without islet cell antibodies with a low risk of developing the disease [4,

5]. In addition, we studied the responses in two control groups, normal non-diabetic subjects and patients with chronic pancreatitis, in order to test if any changes in the twins' B-cell responses were disease specific.

Subjects and methods

We studied non-diabetic identical twins within five years of the diagnosis of Type 1 diabetes in their diabetic twin and a group of patients with chronic pancreatitis as a disease control group. All individuals had had an oral glucose test in order to exclude those with diabetes [6]. In the twin groups monozygosity was established as previously described [4]. The presence of islet cell antibodies was established by testing serum with indirect immunofluorescence on a fresh group 0 cryofixed human pancreas for the presence of both conventional and complement-fixing islet cell antibodies [5]. The titre of each sample was determined by serial doubling dilution. The same pancreas, reagents and incubation conditions gave end-point titres of 32 with the putative islet cell antibody standard (equivalent to 80 Juvenile Diabetes Foundation units) being assessed for the Immunology and Diabetes workshop.

The twins were divided into two groups according to their risk of developing Type 1 diabetes, as defined by the presence of islet cell antibodies [4–6]; group 1, five twins who had complement-fixing islet

Table 1. Mean $\pm$ SD fasting and total concentrations of glucose, insulin and C-peptide in the islet cell antibody positive twins (five patients, six control subjects) after i.v. glucose and i.v. glucagon. There was no differential response to the two stimuli despite a decreased insulin and C-peptide response to both as compared with control subjects

	Twin patients		Control subjects	
	Glucose	Glucagon	Glucose	Glucagon
Glucose				
Fasting (mmol/l)	4.3 $\pm$ 0.6	4.2 $\pm$ 0.8	4.0 $\pm$ 0.4	4.1 $\pm$ 0.4
Total area (mmol $\cdot$ l ⁻¹ $\cdot$ 30 min ⁻¹)	262 $\pm$ 18	52 $\pm$ 6	258 $\pm$ 31	61 $\pm$ 12
Insulin				
Fasting (pmol/l)	143 $\pm$ 31	98 $\pm$ 13	127 $\pm$ 18	111 $\pm$ 28
Total area (pmol $\cdot$ l ⁻¹ $\cdot$ 30 min ⁻¹)	2807 $\pm$ 1778	2186 $\pm$ 1229	7035 $\pm$ 2107 ^a	7490 $\pm$ 2009 ^a
C-peptide				
Fasting (pmol/l)	333 $\pm$ 167	367 $\pm$ 100	300 $\pm$ 133	367 $\pm$ 67
Total area (pmol $\cdot$ l ⁻¹ $\cdot$ 30 min ⁻¹)	7067 $\pm$ 8833	7867 $\pm$ 4867	14300 $\pm$ 7533	21833 $\pm$ 12067 ^a

Twins response vs control subjects: ^a $p < 0.01$

Table 2. Mean $\pm$ SD fasting and total concentration of glucose, insulin and C-peptide in the islet cell antibody negative twins (five patients, six control subjects) after i.v. glucose and i.v. glucagon. There was no differential response to the two stimuli

	Twin patients		Control subjects	
	Glucose	Glucagon	Glucose	Glucagon
Glucose				
Fasting (mmol/l)	4.1 $\pm$ 0.4	3.9 $\pm$ 0.5	4.0 $\pm$ 0.4	4.1 $\pm$ 0.4
Total area (mmol $\cdot$ l ⁻¹ $\cdot$ 30 min ⁻¹)	250 $\pm$ 32	66 $\pm$ 14	262 $\pm$ 27	61 $\pm$ 12
Insulin				
Fasting (pmol/l)	194 $\pm$ 55	95 $\pm$ 42	119 $\pm$ 22	104 $\pm$ 23
Total area (pmol $\cdot$ l ⁻¹ $\cdot$ 30 min ⁻¹)	6405 $\pm$ 5145	6405 $\pm$ 2303	7784 $\pm$ 8248	6573 $\pm$ 1946
C-peptide				
Fasting (pmol/l)	167 $\pm$ 67	300 $\pm$ 33	267 $\pm$ 100	367 $\pm$ 67
Total area (pmol $\cdot$ l ⁻¹ $\cdot$ 30 min ⁻¹)	9133 $\pm$ 5167	16867 $\pm$ 10300	11167 $\pm$ 2367	23933 $\pm$ 11667

cell antibodies, four of whom have subsequently developed Type 1 diabetes (3, 3, 4 and 16 months after these studies), the fifth now has impaired glucose tolerance; group 2, five twins without islet cell antibodies in whom the risk of developing Type 1 diabetes is small, none have developed diabetes to date [7]. The patients with chronic pancreatitis were diagnosed clinically and the diagnosis was confirmed by endoscopic retrograde colangiopancreatography [8]; these patients were studied when clinically well and without symptoms. Each group was compared with unrelated healthy control subjects sought from the local community who were not members of the hospital or medical school community.

The five twins in group 1 were compared with six control subjects of similar age (mean 19.0 $\pm$ 5.1 vs 19.7 $\pm$ 4.8 years), sex (three males in each group) and body mass index (19.0 $\pm$ 2.1 vs 20.5 $\pm$ 1.4 kg/m²). The five twins in group 2 were compared with a further six control subjects who were also similar for age (20.3 $\pm$ 4.4 vs 19.4 $\pm$ 3.2 years), sex (four

males in each group) and body mass index (20.0 $\pm$ 2.4 vs 20.4 $\pm$ 1.5 kg/m²). The group of seven patients with chronic pancreatitis were compared with a group of seven control subjects who were similar in age (50.8 $\pm$ 11.1 vs 49.4 $\pm$ 10.9 years), sex (five males in each group) and body mass index (23.2 $\pm$ 3.1 vs 24.3 $\pm$ 3.7 kg/m²).

Each subject had i.v. glucose and i.v. glucagon given on separate occasions no more than two months apart. Subjects or their parents gave informed consent and the study was approved by the King's College Hospital ethical committee.

Following an overnight fast, subjects were studied supine at least 15 min after a venous cannula was inserted into the antecubital vein under local anaesthesia. Basal blood samples were taken at -10 and 0 min after which either glucose (0.5 g/kg dissolved in a 20% dextrose solution) or glucagon (1 mg dissolved in 1.1 ml of distilled water) were injected i.v. over a period of 2 min. Blood samples were then taken at 3, 5, 7.5, 10, 15, 20, 25 and 30 min, timed from the beginning of the i.v. injection, for the measurement of whole blood glucose, serum insulin and C-peptide.

Whole blood glucose was measured by a glucose oxidase method (Yellow Springs Analyser, Ohio, Yellow Springs, USA). Serum insulin and C-peptide were measured by modifications of double antibody radioimmunoassay methods [8, 9]. All samples were batched so that they were run in the same assays at the same time. i.v. glucose clearance rates were calculated by a method of least squares, using the natural log of the glucose concentration from 15 to 30 min. Total responses were calculated as areas under the curve and above the basal value from time 0 to 30 min following both glucose and glucagon.

Statistical analysis

Results are expressed as the mean $\pm$ SD. The significant variables approximated to a normal distribution in that 66% of the values fell within one SD of their mean. Changes were compared using both a two-tailed Student's *t*-test for paired and unpaired observations and a Wilcoxon's rank sum test; changes were considered significant at $p < 0.05$.

Results

Fasting concentrations

Both groups of twins, the patients with pancreatitis and their respective control subjects had similar fasting concentrations of glucose, insulin and C-peptide before the glucose and glucagon tests (Tables 1, 2 and 3); except that the C-peptide levels both in the immune negative twins and in control subjects for the pancreatitis group were significantly lower before glucose than glucagon ($p < 0.01$, respectively).

Responses to i.v. glucose and i.v. glucagon

Immune positive group of twins and their control subjects. The B-cell responses of these twins to both glucose and glucagon were similar and reduced when compared to their control subjects (Table 1); though the difference for the total C-peptide response to glucose did not reach statistical significance. The insulin area response from 0 to 10 min (first phase, $p < 0.01$), from 10 to 30 min (second phase, $p < 0.05$), and the total response

Fig. 1 a and b. Insulin responses $\pm$ SD in islet cell antibody positive twins (five patients) $\bullet$ and six control subjects $\circ$ to **a** i.v. glucose. * $p < 0.02$ and **b** i.v. glucagon. * $p < 0.02$

($p < 0.01$) were all significantly reduced in twins compared with control subjects (Fig. 1). The B-cells of the control subjects responded similarly to both stimuli.

The B-cells of one patient (M.W.) in the immune positive group three months before he became diabetic showed little response to glucose (total insulin and C-peptide area responses to i.v. glucose were 0 and $183.3 \text{ pmol} \cdot \text{l}^{-1} \cdot 30 \text{ min}^{-1}$, respectively) as compared with total responses to glucagon (4041 and $6333.3 \text{ pmol} \cdot \text{l}^{-1} \cdot 30 \text{ min}^{-1}$, respectively); despite similar fasting glucose levels (5.3 and 4.7 mmol/l) and similar insulin and C-peptide basal levels. This patient's total insulin response to glucagon was only 54% of the mean control response (4041 vs $7490 \text{ pmol} \cdot \text{l}^{-1} \cdot 30 \text{ min}^{-1}$).

Immune negative group of twins and their control subjects. The immune negative twins and their control subjects showed a similar response to the two stimuli (Table 2).

Table 3. Mean $\pm$ SD fasting and total concentrations of glucose, insulin and C-peptide in the pancreatitis patients (seven patients, seven control subjects) after i.v. glucose and i.v. glucagon. There was no differential response between the two stimuli

	Pancreatitis patients		Control subjects	
	Glucose	Glucagon	Glucose	Glucagon
Glucose				
Fasting (mmol/l)	4.5 ± 0.6	4.4 ± 0.5	4.7 ± 0.6	4.4 ± 0.5
Total area (mmol $\cdot \text{l}^{-1} \cdot 30 \text{ min}^{-1}$)	289 ± 47	52 ± 12	282 ± 40	61 ± 12
Insulin				
Fasting (pmol/l)	88 ± 59	146 ± 52	104 ± 24	113 ± 28
Total area (pmol $\cdot \text{l}^{-1} \cdot 30 \text{ min}^{-1}$)	4046 ± 2079	3591 ± 2342	5181 ± 2155	6003 ± 2190
C-peptide				
Fasting (pmol/l)	333 ± 300	467 ± 533	400 ± 133	767 ± 200
Total area (pmol $\cdot \text{l}^{-1} \cdot 30 \text{ min}^{-1}$)	12967 ± 12733	7600 ± 10100	24467 ± 12800	43967 ± 8533^a

Pancreatitis patients vs control subjects: ^a $p < 0.01$

Pancreatitis group and their control subjects. The pancreatitis patients and their control subjects showed a similar response to the two stimuli (Table 3), except that the total C-peptide response to glucagon was significantly lower in the patients with pancreatitis.

Discussion

Auto-immune endocrine diseases, of which Type 1 diabetes is believed to be an example, are associated with specific stimulation, inhibition or destruction of a target cell [10]. The cells involved in these diseases are endocrine cells and it is not surprising that the immune process can target either the signal recognition systems, as in thyrotoxicosis, or the hormone secretion system, as in Hashimoto's thyroiditis. The differential response to glucose and non-glucose secretagogues in patients with Type 1 diabetes raised the possibility that the destructive process leading to Type 1 diabetes preferentially targets the glucose recognition system of the B-cells.

The dose of glucose and glucagon used in this present study gave comparable B-cell responses in three separate groups of normal control subjects and in a group of patients with chronic pancreatitis. The non-diabetic twins we studied without islet cell antibodies were genetically susceptible to Type 1 diabetes but unlikely to develop the disease. These twins had a normal and comparable B-cell response to both glucose and glucagon suggesting that a differential response is not an inherited trait associated with Type 1 diabetes. In contrast, the B-cells of the immune positive group of twins, four of whom have already developed diabetes, had a diminished response to both glucose and glucagon. However, as a group, there was no evidence for a differential response to the two stimuli in the immune positive twins. It therefore appears that the glucose recognition system is not preferentially damaged throughout the pre-diabetic period.

It remains possible that a differential response to glucose and non-glucose stimulants could occur at

some stage of the pre-diabetic period which a cross-sectional study such as ours would not detect. In support of this argument the B-cells of one of our patients (M.W.) did show a zero response to i.v. glucose and an attenuated response (54% of normal) to i.v. glucagon. This patient conforms to the three diabetic and three non-diabetic patients previously described who showed zero or markedly reduced insulin secretion to i.v. glucose with substantially higher, though still subnormal, insulin secretion to i.v. glucagon [2, 3]. The mechanisms which result in such a differential response to glucose and non-glucose stimulants in some pre-diabetic patients are not known. In summary, this study does not support our hypothesis that the initial lesion to the B-cell in Type 1 diabetes preferentially damages the glucose recognition system.

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