The Relationship Between Residual Insulin Secretion and Metabolic Stability in Type 1 (Insulin Dependent) Diabetes

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Summary. The purpose of this study was to investigate whether the great differences in metabolic control between labile and stable insulin dependent juvenile diabetics could be explained by differences in residual pancreatic Bcell function. Nine labile diabetics, ten stable diabetics on one insulin injection a day and nine stable diabetics on two insulin injections a day were investigated during a 24-h period during which they maintained their usual diet and insulin therapy. Serum C-peptide concentrations were measured after removal of proinsulin bound to insulin antibodies. The labile diabetics did not show any significant change in C-peptide concentrations despite great fluctuations in plasma glucose concentrations. In six patients with stable diabetes, C-peptide responses after the main meals could be demonstrated and there was a significant correlation between the concentrations of C-peptide and glucose ($r = 0.85$, $p < 0.001$). The other stable patients, having the same mean plasma glucose concentration and mean amplitude of glycaemic excursions, did not show any C-peptide response. It is concluded that persistent insulin secretion is not a prerequisite for metabolic stability. Severe lability, however, seems to occur only in the absence of residual insulin secretion.

Key words: Type 1 diabetes, labile and stable diabetes, radioimmunoassay of C-peptide, serum C-peptide levels, plasma glucose levels.

The significance of residual insulin secretion, established by the measurement of serum C-peptide levels, for the degree of metabolic control in Type 1 diabetes is not clear. In some studies no better metabolic control was found in patients in whom C-peptide could be demonstrated [1, 2]. Other authors have reported a positive correlation between the degree of metabolic control and C-peptide levels in

groups of patients [3–8], but in individual patients this relationship was not always present. In almost all studies C-peptide immunoreactivity was measured by methods which include proinsulin bound to insulin antibodies. Moreover the measurements were performed in the fasting state or after non-physiological stimuli. In the present study C-peptide was measured after the removal of insulin antibodies in diabetics with stable and labile diabetes during the patients' usual diet and insulin therapy. The aim of the study was to investigate whether the great variation in metabolic stability could be explained by differences in residual insulin secretion.

Patients and Methods

Patients

All patients were Type I (insulin dependent) diabetics, selected as labile or stable patients on the basis of previously established criteria.

The diabetes was considered as labile if all of the following criteria during one year before the start of the study were fulfilled: (1) mean glycosuria >30 g/24 h; (2) the occurrence of high (>20 mmol/l) and low (<10 mmol/l) plasma glucose concentrations at the same time on different days; (3) ketonuria (+ + or + + +) with high glycosuria on at least three visits, without intercurrent illness; (4) occurrence of spontaneous hypoglycaemic episodes; (5) adjustments of the insulin therapy in consequence of these complications; (6) visits to the outpatient clinic at least once a month and good co-operation.

Stable patients had to fulfill all of the opposite criteria during one year: (1) mean glycosuria <30 g/24 h; (2) plasma glucose concentrations always lower than 15 mmol/l; (3) no ketonuria; (4) no hypoglycaemic attacks; (5) a constant insulin dosage; (6) visits to the out-patient clinic at least once in six weeks and good co-operation.

Nine patients with labile diabetes were selected. Their mean ($\pm$ SD) age was 28.9 ± 8.7 years, duration of diabetes 11.1 ± 4.2 years and their mean percentage of ideal body weight was $98.8 \pm 17.9\%$. The mean insulin dosage was 90 ± 37 U/24 h (1.3 ± 0.5 U/kg body weight), given in two injections a day as combinations of long and short acting insulin (usually Ultralente in the morning and Actrapid in the morning and evenings). They had attended the out-patient clinic 12 to 28 times during the year before the study. On 3–28 visits they had complained of unpredict-

Fig. 1. Mean concentration ($\pm$ SEM) of plasma glucose and serum C-peptide in nine labile diabetics during 24 h. Blood samples were taken just before breakfast (B), lunch (L) and dinner (D), $\frac{1}{2}$, 1 and 2 h after these meals, and at 2400, 0400 and 0800 h. S = snack. The insulin injections are indicated by "INS"

able hypoglycaemic attacks and on 4–11 visits ketonuria in combination with glycosuria was found. Due to this the insulin dosage had to be changed 5–19 times. All efforts to achieve better metabolic control had failed without apparent cause.

Nineteen patients with stable diabetes were selected. Ten were treated with one injection a day of long acting insulin. Their mean age was 35.0 ± 9.2 years, duration of diabetes 10.4 ± 7.5 years and mean percentage of ideal bodyweight $104 \pm 5.2\%$. The mean insulin dosage was 34 ± 15 U/24 h (0.5 ± 0.2 U/kg body weight). The other nine patients with stable diabetes were treated with insulin twice a day. Their mean age was 32.4 ± 9.0 years, duration of diabetes 14.2 ± 9.0 years, their percentage of ideal bodyweight $102.2 \pm 8.5\%$ and the insulin dosage was 63 ± 22 U/24 h (0.9 ± 0.3 U/kg body weight). Only the mean insulin dosage was significantly different between the groups.

Design of the Study

On the day of the study, after an overnight fast, the patients came to the ward early in the morning, where they stayed for 24 h. Between the times of blood sampling they were encouraged to walk around. They received their usual diet and insulin therapy. In all patients the diet was given as three main meals and three snacks ($\pm 20\%$ of the calories was supplied as protein, $\pm 40\%$ as fat and $\pm 40\%$ as carbohydrate). Insulin was administered 15 min before breakfast and in patients treated with two injections a day 15 min before the evening meal. Blood for the determination of C-peptide and glucose was taken immediately before the insulin injections,

before the main meals and at 30, 60 and 120 min after the start of the meals, at 2400 and 0400 h, and on the following morning in the fasting state.

Laboratory Methods

C-peptide concentration in serum was measured by radioimmunoassay using synthetic human C-peptide as standard, synthetic human ^{125}I -Tyr-C-peptide as tracer and antiserum M1230, obtained from Novo Industri, Denmark. Insulin antibodies were removed prior to the assay by precipitation with equal amounts polyethylene glycol (PEG 25%) [9]. The final concentration of PEG in serum and in C-peptide standard solution was the same. The detection limit was 0.06 pmol/ml. The PEG treatment of sera did not affect the accuracy of the assay. The intra-assay coefficient of variation for sera with low (0.52 pmol/ml) and high (2.87 pmol/ml) C-peptide concentration was 5.7% ($n = 10$) and 4.4% ($n = 10$). The interassay coefficient of variation was 14.8 and 12.9% for the same sera. The recovery of 0.2–2.0 pmol/ml C-peptide was between 98 and 106%. Serum dilution up to 1:4 did not affect the measurements. In normal lean subjects the mean ($\pm$ SD) fasting C-peptide concentration was 0.53 ± 0.17 pmol/ml (range 0.28–0.86 pmol/ml, $n = 28$).

Plasma glucose was measured by the automated method of Hoffman [10]. The mean amplitude of glycaemic excursions was measured according to Service et al. [11]. Only excursions exceeding one SD of the mean plasma glucose concentration were considered.

Statistics

All results are presented as mean $\pm$ SEM. For statistical evaluation Wilcoxon's test for unpaired data was used.

Results

Labile Diabetics

The mean plasma glucose concentration in the labile diabetics fluctuated widely without a clear relation to meals (Fig. 1). The mean amplitude of glycaemic excursions measured from the individual curves was 17.4 ± 2.8 mmol/l. The fasting C-peptide concentration was 0.19 ± 0.03 pmol/ml. Despite great fluctuations of the glucose concentrations the mean serum C-peptide concentration did not change significantly. In no patient was a variation coefficient of 15% exceeded, the value chosen as the limit for a significant change in concentration.

Stable Patients

In five patients on one insulin injection a day and in eight patients on two injections the mean fasting concentration (0.18 ± 0.02 pmol/ml) did not change significantly during the 24 h (Fig. 2) and at no point was there a significant difference with the mean C-peptide concentration in the labile patients. All the other patients showed a C-peptide response after every main meal (Fig. 2) with mean levels (from 0.25 ± 0.03 pmol/ml in the fasting state) of 0.50 ± 0.08 pmol/ml after breakfast, 0.63 ± 0.04 pmol/ml

after lunch and 0.57 ± 0.09 pmol/ml after dinner. In these subjects a significant correlation existed between the concentrations of C-peptide and glucose ($r = 0.85$, $p < 0.001$). The sum of the increases of C-peptide concentration after each meal was significantly correlated with the mean 24 h plasma glucose concentration ($r = -0.79$, $p < 0.05$). Between the stable diabetics with and without C-peptide response there was no significant difference in mean age, percentage of ideal body weight, mean 24 h plasma glucose concentration and mean amplitude of glycaemic excursions. The six diabetics with C-peptide responses had diabetes for a shorter duration and needed a lower insulin dosage (Table 1).

Fig. 2. Mean concentration ($\pm$ SEM) of plasma glucose and serum C-peptide in the stable diabetics during 24 h with (solid line, $n = 6$) and without (broken line, $n = 13$) a C-peptide response after the main meals. Symbols as in Fig. 1. Only nine patients were treated with an evening injection of insulin (see text)

Discussion

Labile diabetes is characterised by great and unpredictable fluctuations in plasma glucose concentrations both during the day and from day to day, accompanied by a strong tendency to ketosis and to hypoglycaemic reactions, despite all attempts at the best possible treatment [12]. In stable diabetes the opposite is true. It is difficult to define stability and lability quantitatively. Of the criteria that we used each single one is not necessarily a reflection of metabolic (in)stability but we feel that all the criteria together are a valid translation of the descriptive definitions of lability and stability.

It was the purpose of the study to investigate the role of residual insulin secretion in metabolic control during everyday life. Therefore the usual meals of the subjects were chosen as B cell stimulation instead of artificial stimuli, such as glucagon injections. Moreover, the insulin secretion after meals corresponds very well with that after IV glucagon [13, 14].

Before the measurement of C-peptide insulin antibodies were precipitated by PEG 25%. Heding et al. [15, 16] have demonstrated the existence of persistent proinsulin secretion in Type 1 diabetics even when insulin and C-peptide secretion were absent. This proinsulin is bound to insulin antibodies, which delays its clearance. The bound proinsulin reacts with C-peptide antisera and thus must be removed for a reliable C-peptide measurement. This has, however, not been done in studies on residual insulin secretion in labile and stable diabetics so far published.

The labile patients did not show any C-peptide response to changes in plasma glucose concentrations. Nevertheless measurable concentrations were present in all sera. Although a so-called zero value was also found by others [18, 19], this is difficult to explain. It is possible that a small and constant secretion of C-peptide was present. The fact that even during hypoglycaemic periods the C-peptide level did not fall, argues against this theory. Another possibility is that the C-peptide antiserum reacts with free proinsulin, which is not removed by PEG. However,

Table 1. Details of labile and stable diabetics with and without C-peptide responses after meals (mean $\pm$ SEM)

	Labile patients	Stable patients with C-peptide	Stable patients without C-peptide
No of patients	9	6	13
Age (years)	28.9 ± 2.9	32.8 ± 3.7	34.3 ± 2.5
Ideal body weight (%)	98.8 ± 6.0	100.5 ± 2.8	105 ± 1.9
Duration of diabetes (years)	11.1 ± 1.4	5.5 ± 1.4^b	15.2 ± 2.3
Insulin dosage (U/24 h)	90 ± 12^b	29 ± 7^b	56 ± 6
Mean plasma glucose (mmol/l) ^a	17.8 ± 1.6^b	10.0 ± 1.4	12.2 ± 1.0
Mean amplitude of glycaemic excursions (mmol/l) ^a	17.4 ± 2.8^b	7.4 ± 0.8	10.8 ± 1.7

^a Results of 24 h measurements ^b $p < 0.05$ versus other groups

Kuzuya et al. [17] have shown that the free proinsulin concentration is too low to be measured with a C-peptide antiserum that reacts less well with proinsulin than with C-peptide. The cross-reactivity of antiserum M1230 with proinsulin as compared to C-peptide is 11% on a molar base [18]. It has been suggested that C-peptide antiserum reacts with plasma components other than C-peptide and proinsulin [18, 19]. However the exact cause of this remains unclear.

In several other studies labile and stable diabetics were investigated. Shima et al. [5] determined the degree of stability by the magnitude of the standard deviation of ten fasting plasma glucose concentrations. C-peptide immunoreactivity increased after oral glucose and during IV arginine in the stable insulin dependent diabetics but the seven most labile patients did not show a response. Reynolds et al. [3], using the same B cell stimulation, came to similar findings and these authors concluded that the difference between unstable and stable diabetics was partly explained on the basis of persistent insulin secretion in many, if not all, stable diabetics. Yue et al. [6] classified labile or non-labile patients according to their degree of metabolic control. After a 50 g oral glucose load, C-peptide response was absent not only in the labile patients but also in many stable diabetics. In these studies bound proinsulin was not removed before the C-peptide assay. Our results obtained under more normal conditions and after removal of insulin antibodies support the observations of Yue et al. [6].

Although the labile patients did not show any sign of functional B cell activity, this cannot be the only cause of lability, because in 13 of the 19 stable patients C-peptide response was also absent. However in six patients with stable diabetes C-peptide response was evident. In these patients the increases and decreases of plasma glucose were related to meals and there was a significant correlation between the concentrations of glucose and C-peptide, suggesting that the preserved B cell function contributed to the stability. However persistent insulin secretion is not a prerequisite for stability. The absence of persistent B cell function, nevertheless, seems to be associated with severe lability.

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