The effect of acute hyperglycemia on the plasma C-peptide response to intravenous glucagon or to a mixed meal in insulin-dependent diabetes mellitus

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Abstract. The dose-response relationships between prestimulatory blood glucose concentration and the plasma C-peptide responses to stimulation with 1 mg of glucagon iv or a standard mixed meal were studied in 8 C-peptide positive patients with insulin-dependent diabetes mellitus. Hyperglycemia was maintained for 90 min before stimulation using a hyperglycemic clamp technique. Each test was performed at the steady state blood glucose levels ~5, ~12, and ~20 mmol/l. The glucose potentiation of the incremental plasma C-peptide area under the curve at the two levels of hyperglycemia in percent of the area at normoglycemia (median and range) was 343\% (53-1053) (p<0.05) and 341\% (267-1027) (p<0.05) after glucagon and 140\% (76-227) (NS) and 251\% (95-1700) (p<0.05) after the meal. The corresponding relative glucose potentiation of plasma C-peptide 6 min after stimulation with glucagon was 124\% (100-427) (p<0.02) and 144\% (100-209) (p<0.05). There was no significant difference in the degree of glucose potentiation at ~12 or ~20 mmol/l. Furthermore, there was no significant difference in the degree of glucose potentiation of the different estimated values of B-cell function. In conclusion, the plasma C-peptide responses to iv glucagon or to a standard test meal were markedly potentiated by acute hyperglycemia in insulin-dependent diabetes mellitus. No further potentiation was, however, obtained when the prestimulatory blood glucose concentration was raised above 12 mmol/l. These findings contrast those reported in non-insulin-dependent diabetes, where endogenous insulin secretion is potentiated further when the blood glucose concentration is raised to ~20 mmol/l.

More than a decade ago, the plasma C-peptide response to 1 mg glucagon iv was introduced as an estimate of islet B-cell function in insulin-dependent diabetes mellitus (IDDM) (1). The plasma C-peptide response to iv glucagon reaches a maximum after 6 min and is of a similar magnitude as the response to a standard test meal. The plasma C-peptide concentration 6 min after iv injection of 1 mg glucagon has, therefore, gained use as an estimate of residual B-cell function in IDDM (2-10).

By applying the hyperglycemic clamp technique (11), a very pronounced potentiation of B-cell responsiveness to both glucose and other secretagogues is induced by acute hyperglycemia in non-insulin-dependent diabetes mellitus (NIDDM), with a continued potentiation along with an increase in prestimulatory blood glucose concentration from normoglycemia to 20-25 mmol/l (12,13).

In IDDM, the B-cell responsiveness also depends on the concomitant blood glucose concentration (2,3). However, the degree of potentiation induced by different levels of hyperglycemia is not known. Hence, the aim of the present study was to describe the dose-response relationships between different prestimulatory glycemic levels and the plasma C-peptide responses to iv glucagon or to a standard mixed meal in patients with IDDM by use of the hyperglycemic clamp technique.
Patients and Methods

Eight patients with IDDM were studied in the outpatient clinic at the Division of Endocrinology and Metabolism, Århus Amtssygehus. Criteria of IDDM were diagnosis of diabetes before the age of 40 years, weight within 110% of the ideal body weight for the same age and sex (14), and insulin treatment started at diagnosis of diabetes. Only patients with a duration of diabetes below 5 years were considered. Before the study, patients were tested for detectable C-peptide in plasma. None of the patients had intercurrent diseases. The female/male ratio was 5/3. Median age was 27 (range 18-38) years. Duration of diabetes was 1 (½-4) years. Insulin dose was 0.32 (0.22-0.57) IU/kg. Three patients were treated with intermediate acting insulin once daily, 2 patients with intermediate acting insulin twice daily, and 3 patients with multiple injection regimens comprising regular insulin before breakfast, lunch and evening meal and intermediate acting insulin late in the evening. Fasting plasma C-peptide concentration was 0.20 (0.08-0.41) nmol/l and plasma C-peptide concentration 6 min post glucagon was 0.30 (0.12-0.49) nmol/l at prestimulatory euglycemia.

Each patient was examined after an overnight fast on 6 occasions within 7 weeks and with an interval from 1 day to 3 weeks between each test. The last insulin injection was given in the evening or in the morning on the day preceding the investigation. For a given individual, the dosage of insulin prescribed was the same on the day before each experiment. Glucagon or meal stimulation was performed at steady state blood glucose levels of ~5 mmol/l, ~12 mmol/l, and ~20 mmol/l. Since the duration of some of the experiments could be as long as 5 h, the order of the tests was planned to fit patient wishes. However, if patients showed a fasting blood glucose concentration in the range 3.5-6.9 mmol/l, one of the normoglycemic tests were carried out. One patient did not accomplish the glucagon test at the prestimulatory blood glucose concentration of ~20 mmol/l or the meal test at ~12 mmol/l. Informed consent was obtained from all patients. The study was approved by the local ethical committee.

The study protocol is outlined in Fig. 1. The patients were supine with two cannulae placed contralaterally in cubital veins. A priming dose of 36 mg/kg 50% (w/v) glucose for each mmol/l intended increase in blood glucose concentration was given iv (11); hyperglycemia was maintained with a variable infusion of 20% glucose with a volumetric precision pump (IMED 922, IMED Scandinavia, Stockholm, Sweden). Through the contralateral cannula, blood samples were taken every 5 min. After 90 min of stable hyperglycemia, 1 mg of glucagon was injected iv or consumption of the meal was started. The time used for consumption of the meal was not measured. The coefficient of variation of blood glucose values was 0.09 at ~12 mmol/l and 0.12 at ~20 mmol/l. During the tests, glucose infusion was maintained at the prestimulatory rate. Blood samples for determination of plasma C-peptide were taken before induction of hyperglycemia and ~90, ~60, ~30, 0, 3, 6, 9, 15, and 20 min after glucagon injection or ~90, ~60, ~30, 0, 30, 60, and 120 min after the beginning of the meal. Plasma proinsulin concentrations were measured after overnight fasting, after 90 min of stable hyperglycemia, and 6 min after stimulation with glucagon or 120 min after the beginning of the meal. The meal consisted of one boiled egg, half a slice of rye bread with butter, two slices of white bread with butter, 15 g 30% cheese, 20 g jam, 0.2 l skimmed milk, and tea or coffee ad libitum. The meal contained 17 g fat, 50 g carbohydrate, and 23 g protein (1914 kJ). In all patients except one, fasting blood glucose concentrations were within 3.5 to 6.9 mmol/l on at least two occasions. The two tests at normoglycemia could, therefore, be performed directly. One patient was normoglycemic only at one occasion. On this day, stimulation with the meal was performed. Normoglycemia before stimulation with glucagon was obtained by infusion of 2 mIU·kg⁻¹·min⁻¹ regular insulin in isotonic saline (13). After achieving normoglycemia, the insulin infusion was stopped; 90 min later, the stimulation with glucagon was performed.

Prestimulatory plasma C-peptide before glucagon stimulation, plasma C-peptide 6 min after glucagon injection, and the incremental plasma C-peptide area under the curve after stimulation with glucagon or the meal corrected for the prestimulatory level were used as estimates of B-cell function. Relative glucose potentiation was calculated as estimated B-cell function at prestimulatory hyperglycemia in percent of the estimated function at prestimulatory normoglycemia.

Blood glucose concentration was measured by a glucose oxidase method (Glucose analyzer, Yellow Springs Instruments Co, Yellow Springs, Ohio, USA). Plasma C-peptide concentration was measured by the method of Heding (15) with a kit from Novo Research Institute (Bagsvaerd, Denmark). The cross-reactivity with proinsulin on a molar basis was 80%. In our laboratory the within and between coefficients of variation were 0.04 and 0.05. The normal range in 20 fasting non-diabetic subjects was 0.24-0.72 nmol/l. Plasma proinsulin concentration was measured as described previously (12,16). The cross-reactivity with C-peptide was less than 0.4%. The total coefficient of variation of the proinsulin assay was 0.12. The detection limit was 2.5 pmol/l.

Data are expressed as median values with ranges. Wilcoxon's non-parametric ranked-sum test was used when comparing paired data. Spearman's non-parametric ranked-correlation test was used to calculate coefficients of correlation. The level of significance was set at 2α=0.05.

Results

All patients had detectable C-peptide in plasma. Median and ranges of blood glucose and plasma
C-peptide values before and after stimulation with glucagon and the meal are given in Fig. 1. Overnight fasting values of blood glucose and plasma C-peptide were similar on the six study days. A significant increase was found in plasma C-peptide values during continued hyperglycemia (p<0.01). The incremental blood glucose response during the meal at the prestimulatory blood glucose level of ~5 mmol/l was significantly higher than the response at ~20 mmol/l (p<0.05), but not significantly different from the response at ~12 mmol/l. Peak plasma C-peptide after glucagon stimulation was not significantly different from peak plasma C-peptide obtained within 2 h after the start of the meal at each level of prestimulatory blood glucose concentration.

Fig. 2 shows the dose-response relationship between increasing prestimulatory blood glucose concentrations and the concentrations of prestimulatory plasma C-peptide and of plasma C-peptide 6 min after glucagon stimulation. Fig. 3 shows the same dose-response relationship, employing incremental areas under the plasma C-peptide curve after glucagon or the meal as estimates of B-cell function. In Table 1, glucose potentiation of estimated B-cell function is expressed as results at hyperglycemia in per cent of results at normoglycemia. The estimated B-cell function was in all cases significantly potentiated by prestimulatory hyperglycemia (Table 1). However, the estimated B-cell function was in no case significantly more potentiated at ~20 mmol/l than at ~12 mmol/l (Fig. 1, Fig. 2, Table 1). The relative glucose potentiation of the estimated B-cell function at the different blood glucose levels did not differ significantly from each other (Table 1).

The coefficients of correlation between the incremental C-peptide responses to glucagon and
to the meal were 0.81 (p<0.05) at prestimulatory normoglycemia, 0.62 (NS) at a prestimulatory blood glucose concentration of ~12 mmol/l, and 0.81 (p<0.05) at a concentration of ~20 mmol/l.

No differences were found in fasting values of proinsulin on the six study days. The overall median value was <2.5 (<2.5-37.6) pmol/l. This was not significantly different from the median value of 6.3 (<2.5-19.1) pmol/l after 90 min of stable hyperglycemia at ~12 mmol/l or the median 3.6 (<2.5-19.1) pmol/l after 90 min of stable hyperglycemia at ~20 mmol/l. Furthermore, it was not significantly different from the median values 3.0 (<2.5-8.9), <2.5 (<2.5-21.4), and 9.0 (<2.5-29.8) pmol/l obtained 6 min after glucagon stimulation at the prestimulatory blood glucose concentrations ~5, ~12, and ~20 mmol/l, respectively. However, median plasma proinsulin increased significantly 120 min after the beginning of the meal to 10 (6.0-16.2) (p<0.05), 26.0 (13.7-50.9) (<0.01), and 21.8 (<2.5-42.2) pmol/l (p<0.05) at the respective prestimulatory blood glucose values.

The overall median ratio between plasma proinsulin and plasma C-peptide after an overnight fast was 0 (0-0.111). This value was not significantly different from 0.027 (0-0.073) after 90 min of stable hyperglycemia at ~12 mmol/l or from 0.024 (0-0.050) after 90 min of stable hyperglycemia at
Relative glucose potentiation of estimated B-cell function expressed as results at prestimulatory hyperglycemia in percentage of results at prestimulatory normoglycemia (median and ranges).

<table>
<thead>
<tr>
<th>Estimated B-cell function</th>
<th>Prestimulatory blood glucose concentration ~12 mmol/l</th>
<th>Prestimulatory blood glucose concentration ~20 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prestimulatory C-peptide</td>
<td>130 (85-300)*</td>
<td>123 (85-175)*</td>
</tr>
<tr>
<td>C-peptide 6 min post glucagon</td>
<td>124 (100-427)**</td>
<td>144 (100-209)*</td>
</tr>
<tr>
<td>Incremental AUC_{cp} post glucagon</td>
<td>343 (72-1053)*</td>
<td>341 (86-1555)*</td>
</tr>
<tr>
<td>Incremental AUC_{cp} post meal</td>
<td>140 (76-227)</td>
<td>251 (99-1700)*</td>
</tr>
</tbody>
</table>

AUC_{cp} = Plasma C-peptide area.
* p<0.05, **p<0.02. Comparison with result at normoglycemia.

~20 mmol/l. Furthermore, it was not significantly different from the median ratios 0.008 (0-0.022), 0 (0-0.061), and 0.026 (0-0.064) obtained 6 min after glucagon at the prestimulatory blood glucose concentrations ~5, ~12, and ~20 mmol/l, respectively. However, median ratios increased to 0.039 (0.019-0.209) (NS), 0.054 (0.021-0.145) (p<0.05), and 0.049 (0-0.108) (p<0.05) 120 min after the beginning of the meal at the respective prestimulatory blood glucose values. The ratios after the meal did not differ significantly from each other.

Discussion

In the present study, pancreatic B-cell responsiveness was significantly enhanced by an acute elevation in the prestimulatory blood glucose concentration in patients with IDDM. However, no further significant potentiation was obtained when prestimulatory blood glucose concentration was raised above 12 mmol/l. These findings are in contrast to those reported in patients with NIDDM, where a persistent increase is found in the B-cell response to both glucagon and a standard test meal along with an increase in blood glucose concentration from normoglycemia to a level of ~20 mmol/l (12). The more reduced B-cell mass in IDDM seems to be the most likely explanation of the finding that maximal responsiveness is obtained at a lower prestimulatory blood glucose concentration in IDDM than in NIDDM. However, it cannot be excluded that a reduced B-cell sensitivity to glucose in IDDM may also have contributed.

The incremental area under the plasma C-peptide response curve to glucagon showed a median relative glucose potentiation of approximately 340% at each level of hyperglycemia. This pronounced glucose potentiation should be interpreted with caution, since small absolute changes in C-peptide values may result in large percentual changes when related to each other. This is illustrated by the large variability of the relative glucose potentiation of the incremental C-peptide response to glucagon at each level of hyperglycemia (Table 1).

The median potentiation of plasma C-peptide concentration 6 min post glucagon was 120-140% at the two levels of hyperglycemia. This finding is of clinical interest since plasma C-peptide concentration 6 min post glucagon has been used in the discrimination between diabetes types (17-20).

In this study, prestimulatory plasma C-peptide and plasma C-peptide 6 min post glucagon showed a similar degree of glucose potentiation. A similar dependency on the prestimulatory blood glucose concentration agrees well with previous reports suggesting that basal plasma C-peptide and plasma C-peptide 6 min post glucagon basically contain the same information about residual insulin secretion in IDDM (7,20,21).

At prestimulatory normoglycemia, blood glucose as well as plasma C-peptide showed a persistent increase within 2 h after the start of the meal, at which time blood sampling was stopped. In the present protocol, we stopped blood sampling after 2 h because previous studies have shown that peak plasma C-peptide occurs on the average 2 h after the start of a standard meal in patients with IDDM (1). Furthermore, in the same study, the increment-
toral area under the curve to plasma C-peptide within 2 h after the meal correlated closely to the incremental area obtained within 4 h after the start of the meal (r=0.99, P<0.01, N=17). Thus, it is an acceptable assumption that the incremental C-peptide area under the curve within 2 h after the start of the meal is representative of the total area under the curve. The increment in blood glucose concentration during the meal at prestimulatory hyperglycemia was lower than the increment at prestimulatory normoglycemia. The reason for the lower increment in blood glucose concentration probably was the enhanced insulin secretion in combination with an improvement in insulin sensitivity (11). A further explanation could be an enhanced glucosuria during the meal at hyperglycemia. Urinary glucose excretion was, however, not measured during the study. Despite the low increment in blood glucose concentration, prestimulatory hyperglycemia induced a significant potentiation of the plasma C-peptide response to the meal of a similar magnitude as the potentiation of the C-peptide response to glucagon.

During the meal, the ratio between plasma proinsulin and plasma C-peptide showed a significant increase, whereas this was not the case after stimulation with glucagon. Similar findings were reported in patients with NIDDM (12). Thus, the islet B-cells seem to be forced to secrete immature granules during the meal, suggesting that the B-cells are under more stress after the meal than after glucagon.

In conclusion, the plasma C-peptide response to iv glucagon or to a standard mixed meal is potentiated considerably by acutely established hyperglycemia in IDDM. However, no further potentiation seems to be induced when prestimulatory blood glucose concentration is raised above 12 mmol/l.

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