Impaired somatostatin response to orally administered glucose in Type II diabetes entails both somatostatin-28 and -14 and is associated with deranged metabolic control

Mark Gutniak, Valdemar Grill, Arved Roovete and Suad Efendic

Department of Endocrinology, Karolinska Hospital, Stockholm, Sweden

Abstract. We have investigated the effects of hyperglycemia in Type II diabetic patients on the somatostatin response to oral glucose. In these patients hyperglycemia prevailed (11.8 ± 1.4 mmol/l) and was markedly increased to a maximum of 18.9 ± 1.0 mmol/l following the ingestion of 75 g of glucose. The rise in blood glucose following glucose ingestion failed to induce a rise in plasma levels of somatostatin-like immunoreactivity. Biostator-regulated insulin infusion normalized fasting levels of blood glucose and reduced the hyperglycemia following glucose ingestion, i.e. blood glucose now rose from 4.6 ± 0.1 to a maximum of 7.3 ± 0.8 mmol/l. This moderate rise in blood glucose was accompanied by a significant (p < 0.05) rise in somatostatin-like immunoreactivity. Somatostatin-28 and somatostatin-14 were separated using a Sephadex G-50 fine column. Biostator treatment suppressed plasma levels of both peptides during fasting conditions. Treatment was also accompanied by a rise in both peptides during the first hour following glucose ingestion; this rise did not occur in the untreated state. In conclusion: lack of somatostatin response to glucose in non-insulin-dependent diabetes mellitus is associated with deranged metabolic control. Unresponsiveness to glucose entails the secretion of both somatostatin-28 and -14.

Previous studies in man indicate an association between diabetes and D-cell insensitivity to glucose. Ingestion of glucose thus induces a moderate rise in somatostatin-like immunoreactivity (SLI) in non-diabetic subjects (1). This rise was not seen in Type II diabetic subjects with fasting hyperglycemia (2). The oral glucose load, however, did elicit a rise in plasma SLI in subjects with decreased glucose tolerance but with normal fasting blood glucose. This indicates that D-cell insensitivity to glucose is not inherent but evolves only as a consequence of chronic hyperglycemia. These findings are in line with those obtained in animal diabetes in vitro (3–5) or in vivo (6).

The present study aimed to gain further insight into the relation of a diabetic state to apparent D-cell insensitivity to glucose in Type II diabetic subjects. Therefore we studied the effects of Biostator-induced (Miles Laboratories, Elhart, IN) normalization of blood glucose on the SLI response to glucose ingestion. These effects were further analysed by determining levels of somatostatin-28 and -14. The latter determinations provided information as to the site of the insensitive D-cells since the human pancreas mainly contains somatostatin-14, whereas D-cells of the gut contain both peptides (7).

Subjects and Methods

Subjects
Six Type II diabetic patients (4 males, 2 females) participated in the study. Diabetes was diagnosed according to WHO criteria (8). Mean age of the patients was 57.0 ± 2.5 years and body mass index 26.6 ± 1.1 (kg/m²). None of the subjects were markedly obese. Duration of diabetes was 11.7 ± 7.6 years. The mean of the most recent hemoglobin A1c value prior to the study was 7.7 ± 0.2%. All the diabetic subjects were treated by diet alone.

Protocols
The protocols of the study were approved by the Ethical Committee of the Karolinska Hospital. The nature, pur-
pose and possible risks were explained to participants before consent was obtained. Patients with non-insulin-dependent diabetes mellitus were studied as out-patients after an overnight fast. Four to 14 days elapsed between experiments in individual patients.

The patients were studied on two occasions, i.e. during poor metabolic control as well as after normalization of blood glucose by feedback insulin infusion which was delivered by a Biostator as previously described in detail (9). The patients were connected to the Biostator in random order on both occasions. On one occasion the Biostator was used only for monitoring blood glucose levels; on the other occasion the Biostator was also used for delivering insulin. The insulin infusion was started immediately after the first blood sample had been obtained. Normoglycemia was reached within a 20- to 60-min period. The time point when normoglycemia was achieved was designated -30. Thirty min later the patients ingested 75 g of glucose dissolved in 2 ml water/g glucose and flavoured with lemon. Biostator control continued and levels of glucose, free insulin, C-peptide and SLI were followed before and up to 180 min after the ingestion of glucose.

Assays
Blood samples were collected in plastic tubes containing EDTA (0.048 ml; 0.34 mol/l) and Trasylol (1000 kallikrein inhibitor units; Bayer, Leverkusen, FRG) and immediately placed on ice. Samples were centrifugated at 4°C, and plasma was frozen at -20°C. Haemoglobin A1c was measured with fast pressure liquid chromatography (MonoS, Pharmacia). The upper limit (mean +2sd) of normal values was 6.4%. Plasma levels of free insulin were determined by RIA (10, 11), using our own antibodies, and C-peptide by RIA using a commercially available kit (Novo Research Institute, Bagsvaerd, Denmark). All samples from an individual patient were analysed at the same time in each assay.

The procedure of extraction and assay of SLI has previously been described (2). Briefly, SLI was extracted from 2 ml plasma using Vycor glass, eluted from the glass using 1 ml 80% methanol, and after evaporation dissolved in 250 µl assay buffer from which aliquots of 100 µl were assayed in duplicate. The antibody used (R 141E) was raised in our laboratory against somatostatin-14. The limit of detection of somatostatin-14 was 0.5 pg/assay tube. The inter-assay coefficient of variation was 13.5% (N = 8) and the intra-assay coefficient of variation 6% (N = 12).

Somatostatin-14 and -28
Aliquots from samples of all experiments from each protocol were pooled to yield 20 ml plasma. Somatostatin-14 and -28 was extracted from plasma using octadecylsilylsilica cartridges (Sep-Pak, Waters Co, Inc, Milford MA) (12). Eluted and lyophilized somatostatin was dissolved in 1 ml of 0.1 mol/l ammonium acetate and then subjected to gel filtration on a Sephadex G-50 fine column (length 62 cm, diameter 1.6 cm, Pharmacia, Uppsala, Sweden). The column was eluted with 0.1 mol/l ammonium acetate containing 0.025% bovine albumin at a velocity of 30 ml/h. The volume of fractions collected was 1.4 ml. The peak of elution for somatostatin-28 was 99 ml and for somatostatin-14 125.4 ml. Fractions 61-80 were collected for somatostatin-28 and 80-100 for somatostatin-14. The pooled fractions were frozen and lyophilized, dissolved in 2 ml of assay buffer and assayed as above. Somatostatin-14 or -28 served as standard in the assay for the respective peptide. The calculated recovery of added somatostatin-28 (Sigma) and -14 to the column was about 100 per cent.

Statistical methods
Results are given as the mean ± sem. Significance testing was carried out using Student's t-test for paired data. A glucose-stimulated response was calculated as the integrated increase above the basal levels of hormone. These basal levels were calculated and expressed as the mean of measurements during the 30-min period preceding the meal. Correlations were estimated by calculating the Pearson correlation coefficient.

Results
Effects of Biostator treatment: glucose, insulin, C-peptide, SLI
These results are shown in Fig. 1. During fasting conditions, a Biostator-directed infusion of insulin normalized hyperglycemia from 11.8 ± 1.4 to 4.7 ± 0.1 nmol/l (p < 0.005), doubled plasma free insulin (22.9 ± 1.8 vs 44.1 ± 4.1 mU/l, p < 0.05), and markedly decreased C-peptide levels (0.8 ± 0.1 vs 0.3 ± 0.0 nmol/l, p < 0.005). Also SLI levels tended to decrease, although not significantly so (10.0 ± 1.6 vs 5.4 ± 1.1 pg/ml, p < 0.1).

Biostator treatment also affected responses to glucose and hormones following glucose ingestion. The glycemic response calculated as integrated incremental areas under the glucose curves were 950 ± 140 nmol · l⁻¹ · (180 min)⁻¹ in the untreated compared with 273 ± 61 nmol · l⁻¹ · (180 min)⁻¹ in the treated state, p < 0.02 for difference. Areas under the insulin curve were 9-fold lower in the untreated than in the treated state (Fig. 1). Reciprocally, C-peptide levels were higher in the untreated state than during Biostator treatment (78.3 ± 14.1 vs 123.7 ± 7.7 nmol · l⁻¹ · (180 min)⁻¹, p < 0.005).

In the untreated state, only a non-significant tendency was noted for a late rise in SLI levels
Effects of oral glucose (75 g) in subjects with non-insulin-dependent diabetes mellitus with or without Biostator-induced normoglycemia. Mean ± SEM of experiments performed in 6 subjects. --- = untreated, —- = Biostator control. Samples taken at the start of experiments are denoted ⊗ - untreated, ● - before Biostator control.

Following glucose ingestion (636 ± 429 pmol · l⁻¹ · (180 min)⁻¹), no correlation was found between the rise in blood glucose and SLI in individual subjects. Biostator treatment was associated with a moderate but prompt and sustained SLI response to the oral glucose challenge (1416 ± 720 pmol · l⁻¹ · (180 min)⁻¹). Thus, the effect of treatment was significant (p < 0.05).

**Effects of Biostator treatment on levels of somatostatin-28 and somatostatin-14**

These peptides were measured in aliquots of plasma assembled and pooled from all experiments from each of the two protocols employed. Because of this procedure only mean values were obtained. In the untreated state, mean fasting levels of somatostatin-28 and -14 were in the range of values previously reported (13) and were not altered after oral glucose administration. Biostator treatment depressed the mean fasting level of both somatostatin-28 and somatostatin-14 (Fig. 2). Treatment was also accompanied by a marked rise in mean levels of the two peptides already during the first hour following glucose ingestion. The mean absolute levels of somatostatin-28 and somatostatin-14 stimulation with glucose were, however, similar during Biostator treatment and in the untreated state.
Discussion

A previous study showed that D-cells are insensitive to glucose in Type II diabetic subjects (2). The present results show that chronic hyperglycemia is an important cause of D-cell insensitivity. Biostator controlled normalization of blood glucose thus revived the SLI, somatostatin-28, and somatostatin-14 response to glucose. These results suggest that even short-term normalization of blood glucose is sufficient to restore D-cell sensitivity to glucose. An alternative interpretation should, however, be considered. The effect of treatment was to increase incremental rather than absolute levels of somatostatin after glucose. Consequently, the incremental effect of peroral glucose was calculated from a lower baseline in the treated than in the untreated state. It could be argued that D-cells in the untreated state were already maximally stimulated by the prevailing hyperglycemia during fasting conditions and for this reason could not respond to a further elevation of blood glucose following glucose ingestion. Imposing normoglycemia would then relieve the D-cells from stimulation, thereby lowering ‘basal’ levels of somatostatin and allowing a renewed rise in blood glucose to exert a stimulatory effect.

However, our previous studies indicate that a suppressive effect of insulin on fasting levels of SLI is, at least to a major extent, secondary to effects other than a lowering effect on blood glucose. Suppression of SLI levels thus occurred not only during correction of hyperglycemia but also when insulin was infused during continuous normoglycemia (9). The suppressive effect of insulin then could be either a direct or an indirect one. A direct effect on nutrient-stimulated somatostatin secretion has been documented in vitro only at very high concentrations of insulin (14). An indirect effect could result from lowering the levels of hormones, such as glucagon, and of metabolic substrates, such as free fatty acids, which could potentially stimulate the secretion of somatostatin (15). Whether by direct or indirect ways it is logical to assume that the hyperinsulinemia present before and following glucose ingestion exerted a suppressive effect also on the somatostatin response to glucose. This would then result in a less apparent stimulation by glucose than would otherwise be seen.

The effects of Biostator treatment on the glucose-induced SLI response are similar to results in Type II diabetic subjects in whom the SLI response to a mixed meal was tested (9). Also in these former experiments Biostator treatment increased the incremental response but not the absolute levels of
SLI following the meal. The test meal contained only 25% carbohydrates. The meal-induced rise in SLI, thus, is most likely due to stimulation of D-cells by amino acids and/or fatty acids rather than by glucose. These findings could indicate that Biostator-induced normoglycemia does not augment apparent sensitivity selectively to glucose. Note, however, that an SLI response to a mixed meal was present already in the untreated diabetic state, whereas a response to glucose was not. It seems possible that enhancement of a pre-existing response may entail different mechanisms than revival of an absent response.

The separate measurements of somatostatin-28 and -14, albeit limited, indicate that insensitivity to glucose is present in the D-cells of the gut. As already mentioned, the human pancreas contains preponderantly somatostatin-14, whereas the gut contains and probably secretes both somatostatin-14 and -28 (7). Our study shows that neither somatostatin-28 nor somatostatin-14 rose in the untreated diabetic state. Insensitivity of D-cells in the gut should therefore to a major extent be responsible for the absence of an SLI response as measured in peripheral plasma. These findings are in line with the observation in dogs that gut somatostatin determines to a major extent the somatostatin concentration in portal blood (16).

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Dr Mark Gutniak, Department of Endocrinology, Karolinska Hospital, S-104 01 Stockholm, Sweden.