

Suppression of β -Cell Secretion by Somatostatin Does Not Fully Reverse the Disproportionate Proinsulinemia of Type 2 Diabetes

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Disproportionate hyperproinsulinemia is a feature of β -cell dysfunction in type 2 diabetes. It has been hypothesized that this abnormality represents an intrinsic abnormality of the β -cell and/or may result from an increase in β -cell secretory demand. To address this, six patients with type 2 diabetes and six age- and BMI-matched normal subjects received a combined 3-h insulin and somatostatin clamp to decrease β -cell secretory demand. An arginine stimulation test was performed before and at the end of the clamp to measure β -cell peptide release. In keeping with the reduction in secretory demand, C-peptide levels were suppressed by 60–80% during the clamp, as were proinsulin (PI) levels. The arginine-stimulated PI/C-peptide ratio decreased in the diabetic subjects from $4.4 \pm 1.5\%$ before to $1.8 \pm 0.5\%$ after the clamp ($P < 0.01$). This latter ratio was similar to that observed in the normal subjects before the somatostatin infusion ($1.5 \pm 0.3\%$). In the normal subjects, after the clamp the PI/C-peptide ratio had decreased to $0.8 \pm 0.3\%$ ($P < 0.01$). Thus, the postclamp PI/C-peptide ratio in the subjects with type 2 diabetes was elevated compared with that in the normal subjects ($P < 0.05$). Based on these observations, while relief of secretory demand on β -cells by somatostatin decreases the disproportionate elevation in PI levels in patients with type 2 diabetes, the failure to normalize this measure suggests that an intrinsic abnormality of β -cell function exists in subjects with type 2 diabetes that may be aggravated by increased secretory demand. *Diabetes* 53 (Suppl. 3):S22–S25, 2004

Disproportionately elevated fasting and stimulated proinsulin (PI) levels, reflected as an increased ratio of PI and immunoreactive insulin (IRI), are a well established abnormality in type 2 diabetes (1,2). The increase in the fasting PI/IRI is

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IRI, immunoreactive insulin; PI, proinsulin; RIA, radioimmunoassay.
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inversely related to the degree of impairment in β -cell secretory capacity in type 2 diabetes (2) but not with insulin resistance (3). The increased PI/IRI is also related to the severity of diabetes in terms of glycemia (4) but the mechanism responsible for the elevated PI/IRI and the course of this abnormality during the progression of the disease is unknown. In subjects who subsequently developed diabetes, the PI/IRI ratio has been found to be modestly elevated at a stage when fasting glucose levels are only slightly elevated (5), a time when both impaired β -cell function and insulin resistance are present (6).

It has previously been hypothesized that the elevated PI/IRI in type 2 diabetes is an indicator of an intrinsic β -cell lesion associated with inefficient PI processing (7) or is influenced by the increased β -cell secretory demand occurring in type 2 diabetes (8). We approached the question whether either or both of these may be possible explanations for the disproportionate release of proinsulin by suppressing β -cell function in type 2 diabetes subjects using a combined clamp of somatostatin and insulin in order to study the effect of relieving β -cell secretory demand independent of the effect of changing the glucose signal on the cells.

RESEARCH DESIGN AND METHODS

Six subjects with type 2 diabetes and six control subjects participated. All subjects with type 2 diabetes were age- and BMI-matched with control subjects. None of the subjects had clinical or biochemical evidence of cardiac, hepatic, kidney, or thyroid dysfunction. In the subjects receiving hypoglycemic medication, this was discontinued 96 h before the study. The study was approved by the Human Subjects Review Committee at the University of Washington, and all participants gave written informed consent before participating.

All subjects were studied after a 10-h overnight fast. An intravenous line was established in each forearm: one for infusion of insulin, somatostatin, and glucose and one for blood sampling. The latter was wrapped in a heating pad to arteriaize the samples. Thirty minutes after placement of the intravenous lines, three basal samples (–15, –5, and 0 min) were obtained for measurement of glucose, PI, IRI, and C-peptide levels. At time zero, arginine (5 g) was injected intravenously over 30 s and blood samples were drawn at 2, 3, 4, and 5 min after injection. Then, an infusion of constant doses of insulin ($10 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) and somatostatin ($100 \mu\text{g/h}$) was commenced for a period of 180 min. This dose of somatostatin was chosen in order to suppress C-peptide concentrations by 60–80% in the type 2 diabetic subjects. A variable rate infusion of 10% dextrose was also commenced to clamp the plasma glucose level in order to keep the subjects' individual plasma glucose levels constant. Necessary adjustments to the glucose infusion rate were made based on frequent bedside glucose measurements (Beckman, Palo Alto, CA). Five minutes after discontinuation of the somatostatin-insulin infusion, a second dose of arginine (5 g) was injected intravenously and blood samples were drawn at the same intervals as after the first injection of arginine.

PI was assayed by a radioimmunoassay (RIA) measuring both intact PI and its conversion intermediates with 100% efficiency (9). IRI was measured with an RIA that crossreacts 100% with PI and its conversion intermediates (10).

TABLE 1
Characteristics of diabetic and control subjects

	Type 2 diabetic subjects	Control subjects
<i>n</i>	6	6
Age (years)	63.8 ± 1.6	70.8 ± 1.6
Sex (F/M)	3/3	3/3
Fasting plasma glucose (mmol/l)	10.6 ± 1.3*	5.2 ± 0.08
BMI (kg/m ²)	28.6 ± 0.7	30.1 ± 1.2
Duration of diabetes (years)	12.5 ± 3.4	—
Diabetes treatment	5 sulfonylurea, 1 diet	—

Data are means ± SEM. **P* < 0.05.

C-peptide was measured by an RIA (11,12) that exhibits cross-reactivity with intact PI of 13 and 15% with des(31,32)-proinsulin (13).

The incremental PI, IRI, and C-peptide responses to arginine were calculated as the mean of the samples drawn at 2, 3, 4, and 5 min minus the average of the three prestimulus values.

All data are expressed as means ± SEM. Differences were tested by Student's two-tailed *t* test and by Mann-Whitney rank sum test where appropriate. A *P* < 0.05 was considered statistically significant.

RESULTS

The characteristics of the subjects with type 2 diabetes and the control subjects are listed in Table 1. Except for the fasting glucose level, the two groups did not differ for any of these measures.

Fasting levels of PI were elevated in patients with type 2 diabetes (58.2 ± 13.1 vs. 11.8 ± 3.0 pmol/l, *P* < 0.01) as were both IRI (101.0 ± 10.2 vs. 56.3 ± 12.5 pmol/l, *P* < 0.05) and C-peptide (715 ± 35 vs. 533 ± 59 pmol/l, *P* = 0.05) levels. These increases were however disproportionate, so that both the fasting PI/IRI and PI/C-peptide ratios were significantly elevated in the diabetic subjects compared with the control subjects (PI/IRI: 58.3 ± 12.2 vs. 22.3 ± 4.1%, *P* = 0.02; PI/C-peptide: 8.2 ± 1.9% vs. 2.0 ± 0.3%, *P* = 0.01).

Before the clamp, the arginine-stimulated responses of both IRI and C-peptide were significantly reduced in the subjects with type 2 diabetes (IRI: 174 ± 22 vs. 343 ± 49 pmol/l, *P* < 0.05; C-peptide: 465 ± 42 vs. 732 ± 84 pmol/l, *P* < 0.05). In contrast, the arginine-stimulated PI responses tended to be higher in the subjects with type 2 diabetes than in control subjects (21.5 ± 7.4 vs. 10.9 ± 2.9 pmol/l, respectively). Thus, the arginine-stimulated PI/IRI ratio tended to be greater in the diabetic subjects than in the control subjects (13.4 ± 5.0 vs. 3.2 ± 0.8%, respectively; *P* = 0.07). Similarly, the arginine-stimulated PI/C-peptide ratio was increased in the diabetic subjects (4.42 ± 1.48 vs. 1.46 ± 0.34%, *P* = 0.05).

At the end of the 180-min clamp, glucose levels were 10.6 ± 1.5 mmol/l in the diabetic subjects and 5.1 ± 0.1 mmol/l in the control subjects; these levels were similar to those before the clamp was commenced. After 180 min of somatostatin and insulin infusion, C-peptide levels were suppressed from the fasting value by 75% in the diabetic subjects (to 180 ± 24 pmol/l, *P* < 0.001) and by 71% in the control subjects (to 156 ± 38 pmol/l, *P* < 0.001). PI levels were also suppressed by 72% from the fasting level in the type 2 diabetic subjects (to 16.4 ± 4.2 pmol/l, *P* < 0.01) and in the control subjects by 51% (to 5.8 ± 2.2 pmol/l, *P* < 0.01).

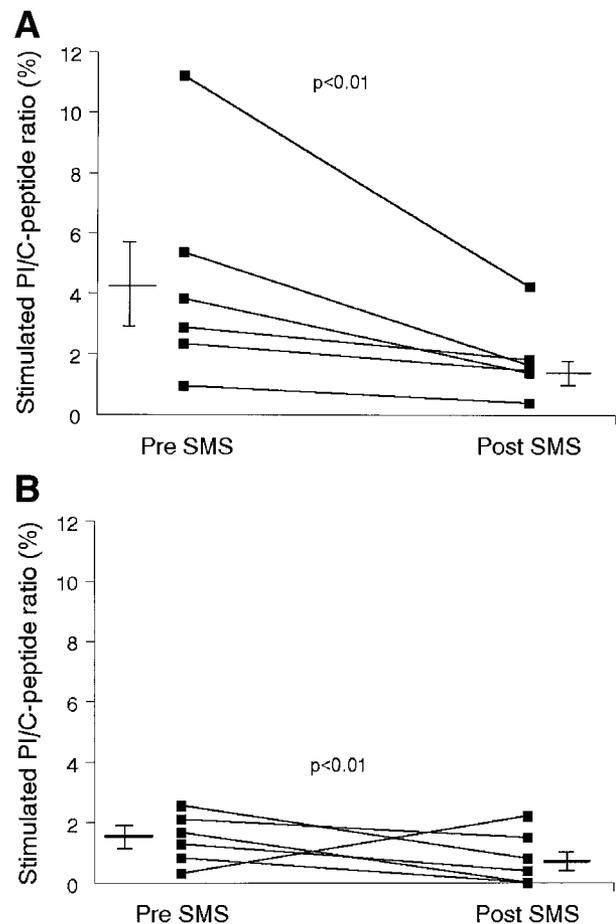


FIG. 1. PI/C-peptide responses to intravenous arginine (5 g) in (A) six patients with type 2 diabetes and (B) six control subjects before and after a 3-h somatostatin-insulin (SMS) clamp during which the glucose level was maintained constant.

As illustrated in Fig. 1, the arginine-stimulated PI/C-peptide responses at the conclusion of the clamp were decreased in all subjects with diabetes from 4.42 ± 1.48% before the clamp to 1.82 ± 0.52% (*P* < 0.01). The arginine-stimulated PI/C-peptide response at the end of the clamp in the diabetic subjects was similar to that in the control subjects before the clamp (1.47 ± 0.34%). However, in the control subjects after the clamp, the arginine-stimulated PI/C-peptide was 0.80 ± 0.30%, which was significantly lower than that in the control subjects before the clamp (*P* < 0.01) and was also significantly lower than that in the diabetic subjects after the clamp (*P* < 0.05).

To determine if suppression of C-peptide and PI levels had occurred after 180 min of somatostatin administration, two subjects with type 2 diabetes were infused with the same somatostatin dose (100 µg/h) along with insulin and glucose for 6 h with repeated arginine stimulation tests performed before and at each hour during the infusion. As depicted in Fig. 2, maximal suppression of the prestimulus as well as arginine-stimulated C-peptide levels had occurred by 180 min and was maintained beyond that time. The same observation was made with PI levels (data not shown).

DISCUSSION

In this cohort of subjects with type 2 diabetes, we observed the well-described finding that fasting PI levels are

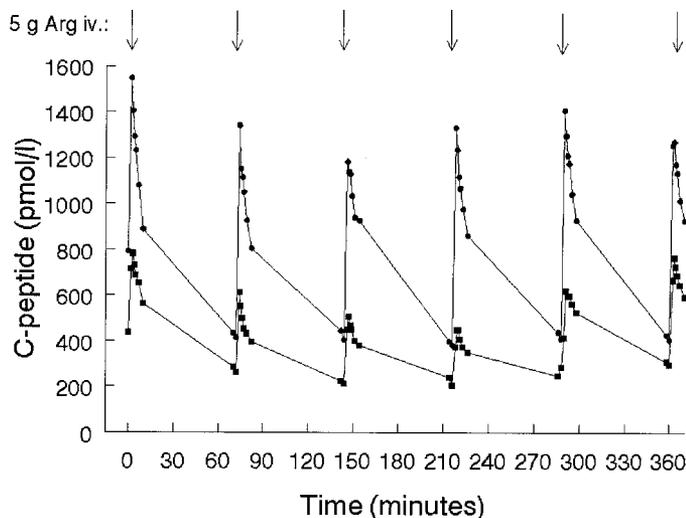


FIG. 2. Time-response study of C-peptide responses to sequential intravenous arginine (5 g) in two patients with type 2 diabetes during a 6-h somatostatin-insulin clamp during which the glucose level was maintained constant.

disproportionately elevated (1,2,9,13). This was true when the PI levels were examined relative to both the fasting IRI and C-peptide levels. The disproportionately elevated acute PI response after arginine stimulation in the subjects with type 2 diabetes is in keeping with the findings of others (2,9,13,14) and supports the concept that the relative release of β-cell products in type 2 diabetes reflects a β-cell secretory abnormality rather than being the result of differences in peptide clearance. When somatostatin was administered to rest the β-cell while maintaining the prevailing glucose levels, we observed that the proportion of PI relative to C-peptide decreased in both the type 2 diabetic and control subjects. However, the ratio remained greater in the diabetic subjects than in the control subjects, in keeping with the continued presence of inefficient proinsulin processing.

The basis for the disproportionate increase in PI in type 2 diabetic subjects has been debated. It has been suggested that it is a manifestation of intrinsic β-cell dysfunction (7) and/or increased secretory demand (8). While the PI/C-peptide ratio decreased in the diabetic subjects, suggesting that decreasing secretory demand may improve the ratio, the fact that this did not suppress to the same level as observed in the control subjects suggests that an abnormality in β-cell function is present. While it would seem possible that this lack of suppression may be due to the differences in the prevailing glucose level, we believe this is unlikely to be the explanation as production of hyperglycemia does not result in a disproportionate increase in PI levels (15,16). It is also unlikely that insulin resistance in the diabetic subjects is responsible for the difference in PI release, as insulin resistance per se has not been shown to result in a disproportionate elevation in PI (13,17–19). Thus, it seems that the disproportionate proinsulinemia observed in type 2 diabetic subjects likely represents an intrinsic β-cell defect that may be aggravated by an increase in β-cell secretory demand.

Examination of the time-dependent nature of the suppression of PI and C-peptide release by somatostatin demonstrated that the prestimulus level at each hour

remained similar during the 6 h of the infusion. This finding strongly suggests that the elevated PI/C-peptide in the diabetic subjects was not due to the fact that the suppression of the β-cell had not occurred at the time that arginine was administered. Further, as the PI/C-peptide ratio did not increase over time with repeated arginine administration, it would appear that if increased secretory demand was important in determining the elevation in the ratio, this degree of demand was insufficient to achieve such a change.

The findings of our study are in keeping with those of Laedtke et al. (20), who infused somatostatin overnight in type 2 diabetic subjects and observed a decrease in the fasting PI/IRI ratio to levels similar to those observed in normal subjects. However, in contrast to the control subjects in our study, their control subjects did not receive a somatostatin infusion. If they had, it is possible that the PI/IRI ratio would have been reduced, as occurred in our study, so that the ratio following β-cell rest in the subjects with diabetes may still have been greater. A number of other studies have also suggested that relieving the increased secretory demand imposed by hyperglycemia does not fully reverse the disproportionate proinsulinemia. Following a period of near-normalization of hyperglycemia achieved by continuous insulin treatment with a pump in type 2 diabetic subjects with marked hyperglycemia (HbA_{1c} levels between 10.7 and 20.5%), PI/C-peptide levels decreased but were not normalized (21). More recently it was reported that following 8 weeks of sulfonylurea or insulin therapy in individuals with milder type 2 diabetes, the PI/C-peptide ratio did not change despite reductions in the fasting plasma glucose level (22).

In summary, we have found that relieving the secretory “drive” on the β-cell in patients with type 2 diabetes by administration of somatostatin is associated with a decrease in the disproportionately increased proinsulinemia, but this does not reduce this measure to the level observed in healthy subjects in whom the β-cell is also rested. This observation supports the hypothesis that there is an intrinsic β-cell abnormality resulting in disproportionately elevated PI levels but also suggests that the continued requirement for insulin secretion that is placed on an impaired β-cell also contributes to this change.

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