Changes in Amylin and Amylin-Like Peptide Concentrations and β-Cell Function in Response to Sulfonylurea or Insulin Therapy in NIDDM

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OBJECTIVE — Amylin, a secretory peptide of β-cells, is the constituent peptide of islet amyloid, which is characteristic of NIDDM, and changes in amylin secretion in response to therapies may influence the rate of production of islet amyloid. The primary objective of this study was to determine whether therapy with sulfonylurea or basal insulin in NIDDM would alter amylin secretion in a way that might affect the formation of islet amyloid.

RESEARCH DESIGN AND METHODS — In a randomized crossover design, eight subjects with NIDDM underwent three 8-week periods of therapy with diet alone, sulfonylurea, or exogenous basal insulin, with evaluation of amylin, amylin-like peptide (ALP), and glucose and C-peptide concentrations, both during fasting and after a standard breakfast. Changes in β-cell function (%β) were assessed, in the basal state by homeostasis model assessment (HOMA) and in the stimulated state by hyperglycemic clamps. Seven nondiabetic control subjects each underwent a meal profile and hyperglycemic clamp.

RESULTS — Both sulfonylurea and insulin therapy reduced basal glucose concentrations compared with diet alone, but neither reduced the increased postprandial glucose increments. Both sulfonylurea and insulin therapy increased basal %β, assessed by HOMA, but only sulfonylurea increased the second-phase C-peptide responses to the hyperglycemic clamp. Sulfonylurea increased time-averaged mean postprandial amylin and ALP concentrations compared with diet alone (geometric mean [1-SD range] for amylin, 4.9 [2.0–11.8] vs. 3.0 [1.4–6.2] pmol/l, $P = 0.003$; for ALP, 16.4 [8.5–31.7] vs. 10.1 [4.9–20.8] pmol/l, $P = 0.001$). Insulin therapy reduced basal ALP concentrations compared with diet alone (2.9 [1.5–5.6] vs. 6.0 [2.6–13.6] pmol/l, $P = 0.03$), but had no effect on postprandial concentrations of amylin (3.0 [1.3–6.5] pmol/l) or ALP (10.0 [5.5–18.1] pmol/l).

CONCLUSIONS — By increasing postprandial concentrations of the constituent peptides of islet amyloid, sulfonylurea therapy might increase the rate of deposition of islet amyloid and thereby accelerate the decline of %β in NIDDM, compared with diet therapy alone.

Amylin, also termed islet amyloid polypeptide, is a normal secretory peptide of β-cells (1). Although its precise physiological function remains unknown, amylin may have pathological significance in that it is the constituent peptide of islet amyloid deposits (2,3), which are a characteristic feature of NIDDM (4). Amyloid deposits form between the islet cells and capillaries and may destroy islet endocrine cells. Although the role of islet amyloid formation in the etiology of β-cell dysfunction in NIDDM has not been fully established, the extent of islet amyloidosis has been shown to be related to the degree of β-cell impairment in spontaneously diabetic monkeys (5) and in humans with NIDDM (6). It has been suggested that once islet amyloid formation has been initiated, polymerization of amylin resulting in the formation of fibrils continues over a period of years, causing progressive deposition of islet amyloid (4). This process may contribute to the progressive worsening of β-cell function (%β), which is a consistent feature of NIDDM (7). Amylin is cosecreted with insulin in response to β-cell secretagogues (8,9), and therapies that alter endogenous insulin secretion are likely to cause changes in amylin secretion. These changes, in turn, may influence the rate of formation of islet amyloid (10), and hence the rate of decline of %β, in NIDDM.

The effect of sulfonylurea of increasing glucose-induced insulin secretion in NIDDM has been well documented in studies that have evaluated insulin secretion at the same plasma glucose levels before and during sulfonylurea therapy (11,12). Studies have suggested that insulin therapy may also increase glucose-induced insulin secretion in NIDDM (13–17) by reducing "glucose toxicity" (18) to the β-cell. Most of these studies, however, have used oral glucose tolerance tests (13–15), with the disadvantage that the resultant variable glucose stimulus, superimposed on variable fasting glucose levels, does not allow clear interpretation of the data.

The primary purpose of this study was to examine the effects of sulfonylurea and basal insulin therapy on fasting and postprandial amylin and amylin-like peptide (ALP) concentrations in subjects with NIDDM to determine whether amylin secretion would be altered in a way that might affect the formation of islet amyloid. To test the concept that insulin therapy might increase glucose-induced insulin secretion in NIDDM, we have also assessed %β in the basal state by homeostasis model assessment (HOMA) (19), and in the stimulated state by hyperglycemic clamping.

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Received for publication 18 June 1997 and accepted in revised form 8 January 1998.

Abbreviations: ALP, amylin-like peptide; %β, β-cell function; CV, coefficient of variation; FPG, fasting plasma glucose; HOMA, homeostasis model assessment.

RESEARCH DESIGN AND METHODS

Subjects

Eight subjects with NIDDM were studied, together with seven nondiabetic control subjects of similar age and obesity (Table 1). The diabetic subjects had all met WHO diagnostic criteria for NIDDM and had been treated from the time of diagnosis with a basal insulin supplement provided by once-daily ultralente insulin, as part of the main randomization of therapy in the U.K. Prospective Diabetes Study (20). The control subjects all had normal fasting plasma glucose (FPG) values and had normal glucose responses to a standard meal-tolerance test. The study was approved by the Central Oxford Research and Ethics Committee, and all subjects gave written informed consent.

Table 1—*Clinical characteristics of subjects*

	Diabetic subjects	Control subjects
n	8	7
Age (years)	63 ± 8	56 ± 7
Sex (M/F)	4/4	3/4
BMI (kg/m²)	31.1 ± 3.7	30.4 ± 2.9
Time from diagnosis (years)	10 ± 3	—
FPG (mmol/l)	11.6 ± 4.1	5.6 ± 0.4
HbA_{1c} (%)	8.6 ± 2.6	5.6 ± 0.5

Data are means ± SD or *n*.

Protocol

In a randomized crossover design, subjects underwent three 8-week treatment periods of their usual diet *1*) without other therapies, *2*) with additional sulfonylurea therapy (gliclazide; Servier, Slough, U.K.), or *3*) with additional basal long-acting insulin (ultralente; Novo Nordisk, Pease Pottage, U.K.). The sulfonylurea was taken as a twice-daily divided dose, and the ultralente insulin was taken as a single daily injection at bedtime. Subjects had FPG measured every 2 weeks, and the dosage of insulin or sulfonylurea (up to a maximum dosage of 160 mg b.i.d.) was increased with the aim of achieving an FPG value <6 mmol/l with no symptoms of hypoglycemia. In the 7th week of each treatment period, after overnight fasting, subjects were admitted for a standard breakfast (397 kcal, 57.1 g carbohydrate). An intravenous cannula was inserted into the distal forearm using local anesthetic, and the arm was heated with a thermoregulated blanket to arterialize the blood samples. Samples were taken to measure glucose, C-peptide, amylin, and ALP levels in the fasting state and for the 3 h after breakfast was started. In the 8th week of each treatment period, after overnight fasting, subjects were admitted for a hyperglycemic clamp. Two intravenous cannulas were inserted using local anesthetic, and arterialized samples were taken from the distal forearm. HbA_{1c} was measured at this visit. After basal blood samples for C-peptide were taken, a glucose bolus was given to raise the blood glucose acutely to 13 mmol/l, and blood glucose was clamped at that concentration for 130 min. Blood glucose was measured every 2 min with a glucose analyzer (Yellow Springs Instruments, Hampshire, U.K.), and the glucose infusion rate was adjusted, using an unbiased, iterative computer program to assess the glucose requirements (21). At 120 min, an intravenous bolus of 5 g L-arginine (Sigma, Dorset, U.K.) was given over a period of 25 s. Blood was drawn continuously by a peristaltic pump during the clamp and arginine infusion, and integrated samples for each time period were used for subsequent laboratory biochemical analyses. Nondiabetic, control subjects attended on two occasions after overnight fasts, and they underwent identical protocols for meal study, hyperglycemic clamp, and arginine infusion.

Biochemical assays

Blood samples were drawn into plastic tubes containing heparin for measurement of glucose and C-peptide, and heparin and aprotinin (Trasylol [2,000 kallikrein inactivator units/ml blood]; Bayer, Leverkusen, Germany) for measurement of amylin and ALP samples, and they were immediately placed on ice. The samples were centrifuged at 4°C. Plasma samples were stored at 4°C, and plasma glucose concentrations were measured within 6 h; plasma samples for other analytes were stored at −20°C until assay. Plasma glucose was determined by a hexokinase method on a Cobas MIRA discrete analyzer (Roche Diagnostica, Herts, U.K.). Plasma C-peptide was assayed by radioimmunoassay (3-h kit for human C-peptide, Novo Nordisk, Bagsvaerd, Denmark) (22). Interassay coefficient of variation (CV) was 4.2% at 1.32 nmol/l. Amylin and ALP were measured with two-site monoclonal antibodies with fluorescent-linked enzyme detection. Amylin was measured with capture antibody F024, which detects unmodified amylin only, and ALP was measured with capture antibody F002, which detects both amylin and additional ALPs (23). These ALPs are higher-molecular-weight glycosylated amylin species that are produced during normal posttranslational processing in the β-cells (24). Minimum detectable amylin and ALP concentrations were 1.9 and 2.7 pmol/l, respectively. Interassay CVs were <15% across the assay range. HbA_{1c} was assayed by high-performance liquid chromatography using a Biorad Diamat automated GHb analyzer (Biorad, Hemel Hempstead, Herts, U.K.), with 4.5–6.2% as the normal range (22).

Data analysis

HOMA of basal %β. Basal (fasting) %β was assessed by the relationship between fasting glucose and C-peptide concentrations, analyzed by HOMA (19). This method uses a computer model based on the known interactions of glucose and insulin in different organs, including the pancreas, liver, and peripheral tissues. For each individual, the model determines the %β that uniquely predicts the measured C-peptide concentration, given the fasting glucose concentration. The %β is expressed relative to values of members of a lean, nondiabetic reference population aged 18–25 years, who were assigned an arbitrary mean value of 100%.

Meal profiles. Postprandial glucose, C-peptide, amylin, and ALP responses were determined in each individual by the time-averaged mean of values for the 3 h after breakfast was started. Incremental postprandial glucose and C-peptide concentrations were determined in each individual by subtraction of the fasting glucose or C-peptide concentrations.

Hyperglycemic clamps. β-Cell responses were also assessed by the second-phase C-peptide response to a hyperglycemic clamp at a blood glucose concentration of 13

mmol/l and by the subsequent response to an intravenous injection of the nonglucose secretagogue arginine at the same glucose concentration. The second-phase C-peptide response consisted of the mean C-peptide concentration from 90 to 120 min of the clamp, calculated from the mean of three 10-min samples, taken by continuous venous sampling. The incremental response to arginine was calculated as the mean C-peptide concentration during the 10-min period after administration of the arginine bolus, measured in five 2-min samples, minus the mean C-peptide concentration during the 10 min preceding administration of the arginine bolus. Both mean C-peptide concentrations were obtained by continuous venous sampling.

Statistical analysis

Positively skewed data were analyzed using log-transformed values. Results are expressed as geometric means (1-SD range), except for the glucose results, which are expressed as means ± SD. Analysis of variance, and paired *t* tests where appropriate, were used to compare the effects of therapies in the diabetic subjects. Unpaired *t* tests were used to compare the data from the diabetic subjects with the nondiabetic control subjects.

RESULTS

FPG and HbA_{1c}

The diabetic subjects treated with diet alone had FPG concentrations (mean ± SD) of 11.6 ± 4.1 mmol/l, which was significantly higher than those of the nondiabetic control subjects (5.6 ± 0.4 mmol/l) (P = 0.004). The FPG concentration was decreased by sulfonylurea therapy to 8.0 ± 2.7 mmol/l (P = 0.003 compared with diet alone) and by insulin therapy to 5.8 ± 1.5 mmol/l (P = 0.004 compared with diet alone). The diabetic subjects treated with diet alone had HbA_{1c} levels of 8.6 ± 2.6%, which was significantly higher than those of the control subjects (5.6 ± 0.5%) (P = 0.01). HbA_{1c} was reduced by sulfonylurea therapy to 7.2 ± 1.5% (P = 0.03 compared with diet alone) and was reduced to an equal extent by insulin therapy, 7.2 ± 1.4% (P = 0.01 compared with diet alone).

Basal %β

Fasting C-peptide concentration (geometric mean [1-SD range]) in the diabetic subjects treated with diet alone was 0.9 (0.6–1.4) nmol/l, which was not significantly different from that in the control subjects (1.0 [0.8–1.3] nmol/l). It was increased by sulfonylurea therapy to 1.1 (0.8–1.5) nmol/l (P = 0.007 compared with diet alone) and was decreased by insulin therapy to 0.5 (0.2–1.0) nmol/l (P = 0.02 compared with diet alone). Basal %β, assessed by HOMA, was 40 (19–84) in the diabetic subjects on diet alone, which was lower than that in the similarly obese control subjects (136 [111–168]) (P = 0.002). Sulfonylurea therapy improved %β to 82 (41–165) (P = 0.0001, compared with diet), and insulin therapy improved %β to 77 (50–119) (P = 0.003 compared with diet alone).

Breakfast glucose and C-peptide profiles

Mean glucose profiles after overnight fasting and in response to a standard breakfast are shown in Fig. 1*A*, and mean incremental glucose responses in Fig. 1*B*. Time-averaged mean glucose concentration (mean ± SD) during the 3-h breakfast profile in the diabetic subjects treated with diet alone was 14.9 ± 4.6 mmol/l, which was significantly higher than that in control subjects, 6.6 ± 0.6 mmol/l (P = 0.001). The glucose concentration was reduced by sulfonylurea therapy to 11.6 ± 3.0 mmol/l (P = 0.007 compared with diet alone) and by insulin therapy to 9.9 ± 1.2 mmol/l (P = 0.006 compared with diet alone). Time-averaged mean incremental glucose response to breakfast in the diabetic subjects treated with diet alone was 3.3 ± 1.0 mmol/l, which was significantly higher than that in control subjects, 1.1 ± 0.7 mmol/l (P = 0.0003). Incremental postprandial glucose responses were not reduced by either sulfonylurea (3.6 ± 0.7 mmol/l) or insulin (4.0 ± 0.9 mmol/l), compared with diet alone.

Geometric mean C-peptide profiles after overnight fasting and in response to a standard breakfast are shown in Fig. 1*C*, and geometric mean incremental C-peptide responses in Fig. 1*D*. The time-averaged mean incremental C-peptide concentration during the breakfast profile in the diabetic subjects treated with diet alone was 0.8 (0.5–1.4) nmol/l, which was significantly lower than that in control subjects, 1.5 (1.0–2.4) nmol/l (P = 0.02). The mean incremental C-peptide concentration was increased by sulfonylurea therapy to 1.2 (0.7–2.0) nmol/l (P = 0.005 compared with diet) and by insulin therapy to 1.0 (0.7–1.6) nmol/l (P = 0.007 compared with diet). The time-averaged mean incremental C-peptide concentration during the first 30 min after breakfast was started was reduced in the diabetic subjects treated with diet alone (0.2 [0.1–0.3] nmol/l) compared with that in control subjects (0.6 [0.3–1.0] nmol/l) (P = 0.001) and was not significantly improved by either sulfonylurea or insulin therapy.

Plasma amylin and ALP responses

Geometric mean amylin profiles after overnight fasting and in response to a standard breakfast are shown in Fig. 2*A*, and geometric mean ALP responses in Fig. 2*B*. Comparison of amylin concentrations in the fasting state was not performed because most of the diabetic subjects treated with either diet alone or insulin had fasting concentrations below the minimum detectable concentration of the assay. Fasting ALP concentration in the diabetic subjects treated with diet alone was 6.0 (2.6–13.6) pmol/l, which was not significantly different from that in control subjects, 7.4 (4.6–11.9) pmol/l. Sulfonylurea therapy did not significantly alter the fasting ALP concentration (6.4 [3.0–14.0] pmol/l), but insulin therapy reduced it to 2.9 (1.5–5.6) pmol/l (P = 0.03 compared with diet alone).

Time-averaged mean amylin concentration during the breakfast profile in the diabetic subjects treated with diet alone was 3.0 (1.4–6.2) pmol/l, which did not differ significantly from that in control subjects 6.1 (3.5–11.5) pmol/l (P = 0.06). Sulfonylurea therapy increased it to 4.9 (2.0–11.8) pmol/l (P = 0.003 compared with diet alone), but insulin therapy did not alter it (3.0 [1.3–6.5] pmol/l). Time-averaged mean ALP concentration during the breakfast profile in the diabetic subjects treated with diet alone was 10.1 (4.9–20.8) pmol/l, which was not significantly different from that in control subjects, 17.1 (9.8–29.8) pmol/l (P = 0.14). Sulfonylurea therapy increased the time-averaged mean ALP concentration to 16.4 (8.5–31.7) pmol/l (P = 0.001 compared with diet alone), but insulin therapy did not significantly alter it (10.0 [5.5–18.1] pmol/l).

The ratios of amylin or ALP to C-peptide were assessed to evaluate whether relative secretion of the β-cell peptides was similar in all therapy groups. The fasting ALP–to–C-peptide ratios, and the postprandial amylin–to–C-peptide and ALP–to–C-peptide ratios, were similar in the control subjects and diabetic subjects treated with diet alone and were not significantly altered by either sulfonylurea or insulin therapy.

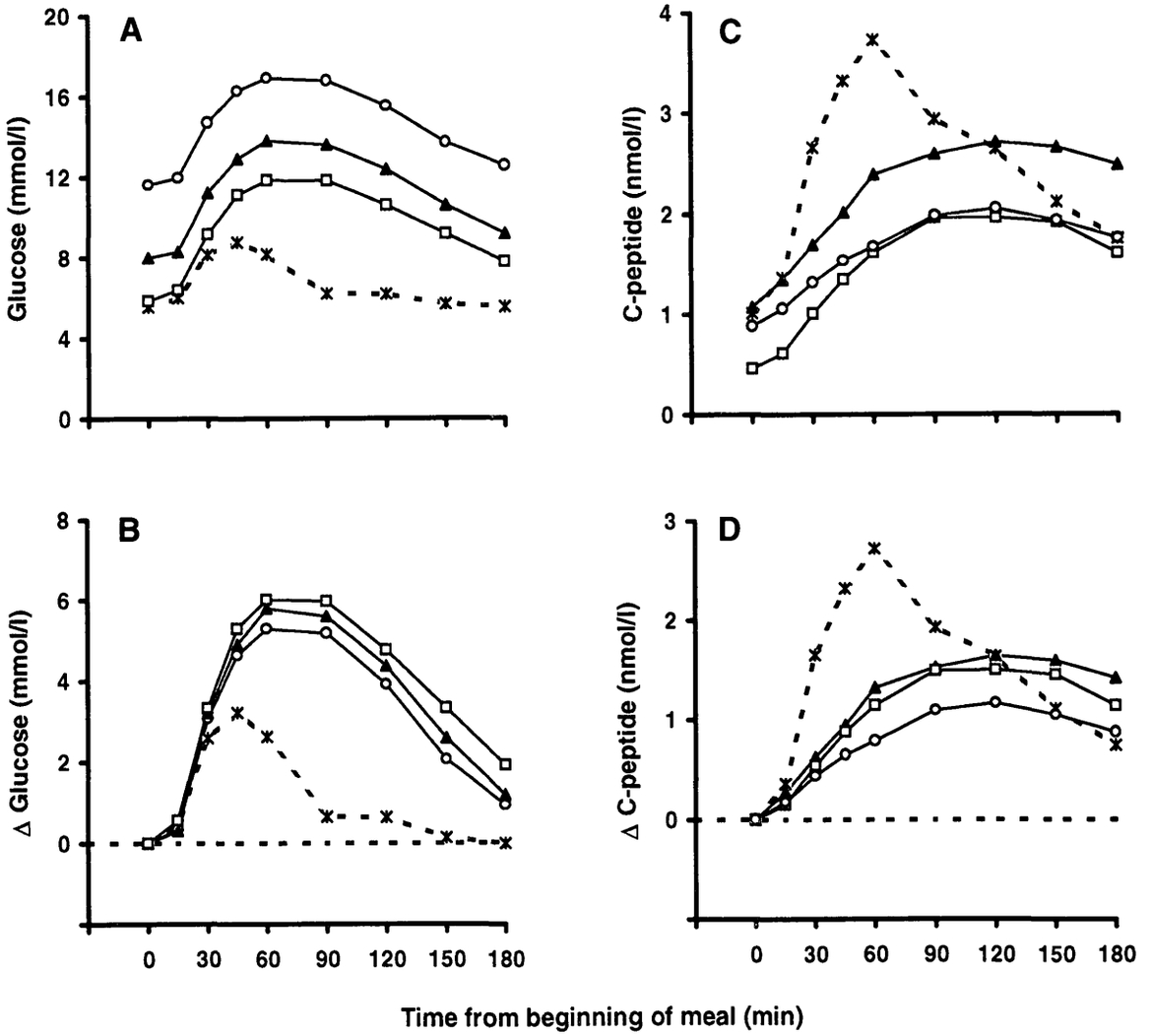

Figure 1—*Measurements of mean plasma glucose concentrations (A), mean incremental glucose concentrations (B), geometric mean C-peptide concentrations (C), and geometric mean incremental C-peptide concentrations (D) in response to a standard breakfast in diabetic subjects treated with diet alone (—○—), sulfonylurea (—▲—), or insulin (—□—), compared with nondiabetic control subjects (- - -✱- - -).*

Hyperglycemic clamps

Hyperglycemic clamps were performed at a blood glucose concentration of 13 mmol/l, which resulted in a subsequent mean laboratory plasma glucose concentration (for all subjects) of 16 ± 1 mmol/l. The geometric mean C-peptide profiles during the hyperglycemic clamps are shown in Fig. 3. The second-phase C-peptide response to glucose was impaired in the diabetic subjects treated with diet alone (1.3 [0.7–2.5] nmol/l) compared with the nondiabetic control subjects (4.2 [2.3–7.5] nmol/l) (P = 0.002). This response was increased by sulfonylurea to 2.0 (1.1–3.7) nmol/l (P = 0.001 compared with diet alone) but was not significantly changed by insulin therapy (1.4 [0.8–2.4] nmol/l). The arginine-stimulated first-phase incremental C-peptide response was impaired in the diabetic subjects treated with diet alone (0.8 [0.5–1.4] nmol/l) compared with nondiabetic control subjects (3.8 [1.6–9.3] nmol/l) (P = 0.003). This response was not significantly changed by either sulfonylurea (1.0 [0.5–1.9] nmol/l) or insulin therapy (0.7 [0.4–1.3] nmol/l).

CONCLUSIONS — Amylin and ALP concentrations paralleled C-peptide concentrations in both diabetic and control subjects, and the close cosecretory pattern was not altered by either sulfonylurea or insulin therapy. Sulfonylurea therapy increased postprandial amylin and ALP concentrations compared with diet alone. This finding raises the possibility that therapy with sulfonylurea, by increasing concentrations of the constituent peptides of islet amyloid, may lead to greater rate of deposition of islet amyloid and faster decline in %β in NIDDM than therapy with either diet alone or insulin. Although in the U.K. Prospective Diabetes Study, the rate of decline in %β in subjects treated with sulfonylurea appeared to be similar to that in subjects treated with diet therapy alone (7), that analysis was performed in patients who were maintained on diet alone or sulfonylurea therapy for 6 years and did not include comparison of groups that were specifically matched at baseline. Analysis comparing the rate of decline in %β between diet- and sulfonylurea-treated groups that are matched at baseline will indicate whether sulfonylurea-induced increases in amylin and ALP concentrations might be pathophysiologically significant.

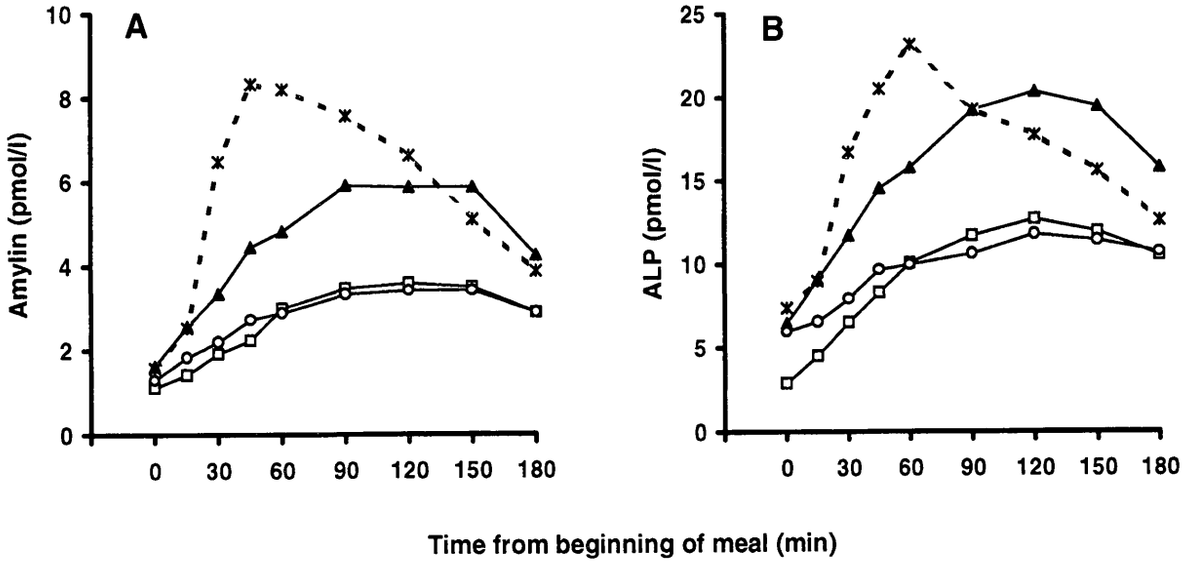

Figure 2—*Measurements of geometric mean amylin concentrations (A) and geometric mean amylin-like peptide (ALP) concentrations (B) in response to a standard breakfast in diabetic subjects treated with diet alone (—○—), sulfonylurea (—▲—), or insulin (—□—), compared with nondiabetic control subjects (- - -✱- - -).*

Both sulfonylurea and basal insulin therapy reduced postprandial glucose concentrations, but this finding merely reflects reduction in basal glucose concentrations because neither therapy reduced the exaggerated glucose increments that followed the standard meal. Although both therapies appeared to increase C-peptide concentrations relative to glucose concentrations after the standard meal, the C-peptide increments measured during the first 30 min after breakfast was started were substantially impaired in the diabetic subjects treated with diet alone compared with the increments measured in the control subjects and were not increased by either therapy. Although the first-phase C-peptide responses to intravenous glucose were not assessed in this study because of the differences in fasting glucose concentrations among therapies, neither sulfonylurea nor insulin increased first-phase C-peptide responses to intravenously administered arginine. The failure to reduce postprandial glucose increments may reflect the lack of increase of early-phase insulin responses, which are thought to have an important role in postprandial glucose control (25,26).

The failure of these therapies to reduce postprandial glucose increments in NIDDM has been observed previously (12,14,16,27), and a similar inability of metformin to lower glucose increments in the oral glucose tolerance test has also been demonstrated (28). These observations have important implications from both a clinical point of view, in that addition of therapy designed to reduce postprandial glucose increments—such as acarbose—has a substantial additive effect on reduction of overall glycemia in NIDDM (29), and a pharmaceutical point of view, in that new therapies that would target both basal glucose concentrations and postprandial glucose increments would be of great advantage.

Sulfonylurea therapy increased glucose-induced insulin secretion in the basal state, as measured by HOMA analysis, and also

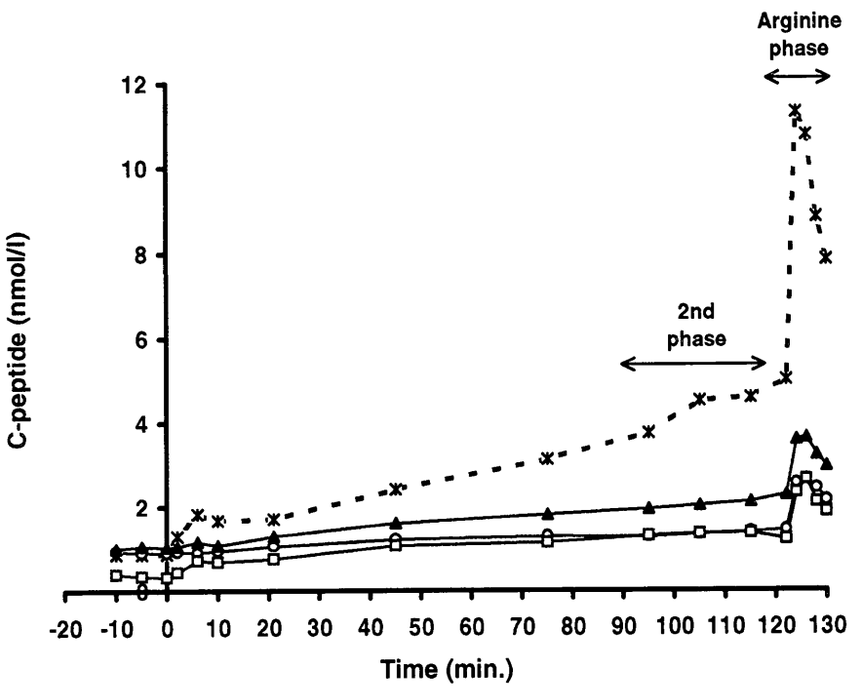

Figure 3—*C-peptide profiles (geometric means) during hyperglycemic clamp at 13 mmol/l in diabetic subjects treated with diet alone (—○—), sulfonylurea (—▲—), or insulin (—□—), compared with nondiabetic control subjects (- - -✱- - -). The clamp was commenced at 0 min, and the arginine was administered at 120 min.*

increased second-phase C-peptide responses to the hyperglycemic clamp. This observation suggests that sulfonylurea therapy increased both sensitivity and responsiveness of glucose-induced insulin secretion, i.e., it both shifted the insulin-glucose dose-response curve to the left (equivalent to a reduced K_m) and increased the maximal insulin response to glucose. Exogenous insulin therapy reduced basal C-peptide concentrations compared with diet alone, but HOMA analysis of the basal C-peptide concentrations relative to the substantially reduced fasting glucose concentrations induced by insulin showed that insulin therapy had increased glucose-induced insulin secretion in the basal state to a level similar to that produced by sulfonylurea. This finding supports the concept that insulin therapy can increase glucose-induced insulin secretion in NIDDM. The precise impact of insulin therapy on glucose-induced insulin secretion in NIDDM is complex, however, because the second-phase C-peptide responses to the hyperglycemic clamp were not increased, suggesting that insulin therapy had shifted the insulin-glucose dose-response curve to the left but had no effect on the maximum insulin response to glucose.

In conclusion, basal insulin therapy in subjects with NIDDM decreased basal ALP concentrations but did not affect postprandial amylin and ALP concentrations compared with diet therapy alone. On the other hand, sulfonylurea therapy increased postprandial amylin and ALP concentrations compared with diet therapy alone. This increase in the concentrations of the constituent peptides of islet amyloid in NIDDM may be disadvantageous in the long term.

Acknowledgments — The study was supported by an academic grant from Novo Nordisk, and the kits for amylin and ALP measurement were provided by Amylin Pharmaceuticals. J.R. is a recipient of a Baron Hartley Scholarship from the University of Cape Town and a United Kingdom Overseas Research Studentship.

We are grateful to Pauline Sutton, Hilary Davies, and Dr. Sue Manley for the biochemical assays and to Dr. Anne Clark for helpful comments.

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