The Amylin Analog Pramlintide Improves Glycemic Control and Reduces Postprandial Glucagon Concentrations in Patients With Type 1 Diabetes Mellitus

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To explore further the effects of the human amylin analog pramlintide on overall glycemic control and postprandial responses of circulating glucose, glucagon, and metabolic intermediates in type 1 diabetes mellitus, 14 male type 1 diabetic patients were examined in a double-blind, placebo-controlled, crossover study. Pramlintide (30 μg four times daily) or placebo were administered for 4 weeks, after which a daytime blood profile (8:30 AM to 4:30 PM) was performed. Serum fructosamine was decreased after pramlintide (314 ± 14 μmol/L) compared with placebo (350 ± 14 μmol/L, $P = .008$). On the profile day, the mean plasma glucose (8.3 ± 0.7 *v* 10.2 ± 0.8 mmol/L, $P = .04$) and postprandial concentrations (incremental areas under the curve [AUCs] from 0 to 120 minutes) were significantly decreased during pramlintide administration ($P < .01$ for both) despite comparable circulating insulin levels (359 ± 41 *v* 340 ± 35 pmol/L). Mean blood glycerol values were reduced (0.029 ± 0.004 *v* 0.040 ± 0.004 mmol/L, $P = .01$) and blood alanine levels were elevated (0.274 ± 0.012 *v* 0.246 ± 0.008 mmol/L, $P = .03$) after pramlintide versus placebo. Blood lactate concentrations did not differ during the two regimens. During pramlintide administration, the AUC (0 to 120 minutes) for plasma glucagon after breakfast was diminished ($P = .02$), and a similar trend was observed following lunch. In addition, peak plasma glucagon concentrations 60 minutes after breakfast (45.8 ± 7.3 *v* 72.4 ± 8.0 ng/L, $P = .005$) and lunch (47.6 ± 9.0 *v* 60.9 ± 8.2 ng/L, $P = .02$) were both decreased following pramlintide. These data indicate that pramlintide (30 μg four times daily) is capable of improving metabolic control in type 1 diabetics. This may relate, in part, to suppression of glucagon concentrations. Longer-term studies are required to ascertain whether these findings are sustained over time.

AMYLIN is a 37–amino acid peptide that is cosecreted with insulin.[1] Its possible role in human physiology is sparsely defined, but recent studies in animals and humans indicate that both the native peptide and the human amylin analog pramlintide ($Pro^{25,28,29}$-human amylin, previously referred to as AC137) are capable of influencing metabolism. In type 1 diabetes mellitus, acute pramlintide infusion has been demonstrated to attenuate postprandial glycemic excursions.[2] Moreover, glycemic control has been shown to improve during 14 days[3] and 28 days[4] of treatment with pramlintide. Acute studies in humans have failed to demonstrate altered insulin sensitivity during exposure to amylin or the amylin analog.[5-7] The reduced postprandial glycemic excursions during pramlintide treatment are probably due in part to a delay in nutrient delivery to the peripheral circulation,[8,9] amylin being a potent inhibitor of gastric emptying.

Recently, it has also been shown that amylin, like insulin, inhibits the glucagon response to arginine in rats in a dose-dependent manner.[10] Conversely, administration of amylin antagonists has been found to result in hyperglucagonemia.[11] This observation could have therapeutic implications because diabetes mellitus is associated with hyperglucagonemia in both the fasting and the postprandial state.[12-14] The combination of insulin deficiency and glucagon excess led more than two decades ago to the so-called bihormonal hypothesis of diabetes, postulating that both hormones contribute to the metabolic derangement of diabetes mellitus.[15,16]

The present study was undertaken to elucidate further the effect of 4 weeks of amylin-analog administration on glycemic control in patients with type 1 diabetes mellitus and, moreover, to assess its possible impact on circulating concentrations of glucagon and intermediary metabolites.

SUBJECTS AND METHODS

Subjects

Fourteen male type 1 diabetic patients participated in the study. The mean age was 36.6 years (range, 23.6 to 53.2), body mass index 23.4 kg/m^2 (range, 19.7 to 26.8), diabetes duration 13.3 years (range, 3.3 to 34.0), basal C-peptide less than 0.33 nmol/L, daily insulin requirement 46.4 U (range, 18 to 70), baseline hemoglobin A_{1C} (HbA_{1c}) 8.6% (range, 7.3% to 9.9%; reference range, 4.3% to 6.1%), and blood pressure 123/78 mm Hg (range, 138 to 110/85 to 65 mm Hg). Background retinopathy was present in four patients and intermittent microalbuminuria in one patient; apart from this, no long-term diabetic complications were recorded. Insulin therapy was administered to all patients as fast-acting insulin (insulin Actrapid; Novo-Nordisk, Copenhagen, Denmark) before each major meal (thrice daily) and long-acting insulin (insulin Insulatard; Novo-Nordisk) at 10:00 PM. A medical screening including physical examination, biochemical screening, and electrocardiogram revealed no evidence of significant concomitant diseases. No medication apart from insulin was used. The experimental protocol was approved by the Ethics Committee of the County of Aarhus and the Health Authorities of Denmark. All subjects provided written informed consent.

Study Design

The design was double-blind and placebo-controlled. Participants randomly underwent two treatment periods each lasting between 26 and 28 days (referred to in the following as a 4-week period), during which they were treated with subcutaneous injections of placebo or Pramlintide at a dose of 30 μg four times per day. On the last day of each

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treatment period, the patients were admitted to the Clinical Research Unit, Department of Medicine M (Diabetes and Endocrinology), Aarhus Kommunehospital, for a daytime profile under standardized conditions. The two treatment periods were separated by a washout period of 3 to 5 weeks. Three days before the profile day, all patients were instructed to consume a weight-maintaining diet containing 300 g carbohydrate, and none engaged in heavy physical exercise in the same period. None had a history of infectious disease within the 2 weeks prior to the study or during the study periods.

Treatment Periods

Following inclusion, the participants were interviewed by a dietician to establish baseline energy intake and baseline weight was recorded. They were asked to maintain their diet unchanged throughout the study periods. The patients were instructed to administer study medication approximately 15 minutes before each meal (breakfast, lunch, dinner, and evening snack). Insulin dosage was adjusted by a physician according to the occurrence of hyperglycemic or hypoglycemic episodes. Participants were also given insulin and glucose diaries and asked to perform home measurements of blood glucose four times per day (before each meal and at bedtime) for 3 successive days during the first week of treatment and 1 day each week during the remaining 3 weeks of treatment, with the last recorded day immediately preceding the study day. Patients were contacted by a physician at least once per week during the 4-week treatment period. They were interviewed with respect to administration of study medication, possible side effects, and glycemic control based on home measurements of blood glucose. If necessary, the insulin dosage was adjusted in collaboration with the physician. Finally, the patients were requested to record diet intake on 2 weekdays and 1 day on the weekend during the third week of treatment, and these data were evaluated by a dietician. Irrespective of possible changes in the total insulin dosage during treatment, patients were asked to keep the dose of long-acting insulin on the last day prior to the profile day constant during the two treatment periods.

Daytime Profile

At 7:30 AM, patients were admitted to the Clinical Research Unit for a daytime profile (8:30 AM to 4:30 PM) under standardized conditions. The diet was prepared by a dietician and represented a typical diet for a type 1 diabetic male, identical for all patients. The energy content of breakfast (ingested between 8:30 and 8:45 AM) was 2,067 KJ (energy %, protein 15, fat 38, and carbohydrate 47), lunch (12:30 to 1:00 PM) contained 2,238 KJ (energy %, protein 16, fat 35, and carbohydrate 49), and the afternoon snack (3:30 to 3:45 PM) contained 1,255 KJ (energy %, protein 7, fat 31, and carbohydrate 62). Breakfast was mainly based on rapidly absorbed carbohydrate (ie, orange juice, milk, and fruit) and lunch was primarily based on slow-release carbohydrate (ie, bread and vegetables). Between meals, patients were only allowed to drink tap water except for a cup of coffee served at 10:30 AM. However, if hypoglycemia occurred, an oral carbohydrate supply was administered to restore normoglycemia. During the day, patients were given their usual study medication (placebo or pramlintide) administered 15 minutes before meals. We aimed to administer identical insulin doses prior to the meals on the 2 profile days, as well as on the evening before the profiles; ie, the insulin doses given during the first examination were mimicked on the second examination unless contraindicated by the current glycemic level.

Before the profiles, weight was recorded and an intravenous catheter was positioned in an antecubital vein for blood sampling. Blood for determination of plasma glucose was drawn at 8:30, 8:40, 8:50, 9:00, 9:10, 9:30, 10:30, and 11:30 AM and 12:30, 1:00, 1:30, 2:30, 3:30, and 4:30 PM, and blood for measurement of plasma glucagon, serum insulin, growth hormone (GH), and nonesterified fatty acids (NEFAs), and blood alanine, lactate, 3-hydroxybutyrate (3-OHB), and glycerol was collected at 8:30 AM and every hour throughout the study period. A baseline blood sample for determination of HbA_{1c} and fructosamine was obtained at 8:00 AM.

Analytical Methods

Plasma glucose was determined in duplicate immediately after sampling (Beckman Instruments, Palo Alto, CA). Serum free insulin was determined by an automated immunoassay system with fluorescence detection in a one-step sandwich assay followed by a competitive immunoenzymatic assay (TOSOH kit; TOSOH, Foster City, CA), and serum GH was assayed using an immunofluorometric sandwich assay with two monoclonal antibodies (Delfia human GH kit; Wallac Oy, Turku, Finland). Plasma samples to be analyzed for glucagon were stored at −20°C with aprotinin and EDTA (300 μL aprotinin and 30 μL EDTA added to 3 mL blood). Samples were collected on an ice bath and frozen immediately until analysis. All samples from each participant were analyzed in the same assay. Measurements were performed by radioimmunoassay as previously described by Ørskov et al[17] using wick-chromatography, except polyethylene glycol was used for separation prior to determination and plasma was extracted with ethanol. This assay is specific for pancreatic glucagon and does not measure intestinal proglucagon-derived molecules. The intraassay coefficient of variation was less than 2.0% (n = 45) at a plasma level of 50 ng/L. The serum NEFA level was measured by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany), and blood lactate, glycerol, 3-OHB, and alanine were assayed using the Cobas Bio centrifugal analyzer (Roche Products, Welwyn Garden City, UK) with a fluorometric attachment.[18] Serum leptin was assayed using a commercial kit (Linco Research, St Charles, MO), HbA_{1c} was determined by high-performance liquid chromatography (BIORAD Laboratories, Munich, Germany), and fructosamine was assayed by a reduction technique (Roche, Basel, Switzerland).

Statistical Analyses

All data are presented as the mean ± SEM unless otherwise indicated. Two-period crossover ANOVA (or two-period Wilcoxon rank-sum test, when appropriate) was used for comparison of data between treatment periods. Furthermore, differences in dynamic changes during the profile day between treatment periods were examined using a two-way ANOVA for repeated measures. AUCs were calculated using the trapezoidal rule, and incremental AUCs were calculated by subtracting the baseline value (ie, the value immediately prior to the meal) from the AUC. All statistical analyses were performed using SAS Version 6.11 (SAS Institute, Cary, NC).

RESULTS

Four-Week Period

Energy intake at screening was estimated to be 10,736 ± 675 KJ (energy %, carbohydrate 48 ± 2, protein 15 ± 1, and fat 35 ± 2), and this did not change following 3 weeks of pramlintide *v* placebo (11,182 ± 720 *v* 11,568 ± 819 KJ, $P = .66$; energy %, carbohydrate 46 ± 3 *v* 47 ± 3, protein 17 ± 1 *v* 17 ± 1, and fat 34 ± 3 *v* 34 ± 3). Body weight at screening was 74.9 ± 2 kg. However, despite apparently similar energy intake, weight loss was observed following both pramlintide and placebo (−2.3 ± 0.3 *v* −1.3 ± 0.4 kg), although this weight loss did not differ between treatment periods ($P = .12$). HbA_{1c} was 8.6% ± 0.3% and did not change significantly following treatment (7.9% ± 0.3% *v* 8.2% ± 0.3%, $P = .32$, pramlintide *v* placebo). By contrast, the level of fructosamine (n = 13) was significantly lower following pramlintide versus placebo (314 ± 14 *v* 350 ± 14 μmol/L, $P = .008$).

Table 1. Daily Insulin Requirements During the Study(mean ± SEM)

Study Week	Insulin (U)		
	Pramlintide	Placebo	*P*
1	46.3 ± 3.6	48.2 ± 4.2	.38
2	45.6 ± 3.4	46.6 ± 4.3	.73
3	46.3 ± 3.4	50.2 ± 4.0	.02
4	46.1 ± 3.5	49.5 ± 3.5	.02

The insulin requirement was higher during placebo compared with pramlintide during the last 2 weeks of the treatment periods (Table 1). The mean preprandial blood glucose concentrations based on home glucose monitoring did not differ between the two treatment regimens (Table 2).

Daytime Profile

Plasma glucose and serum insulin. The mean daytime plasma glucose was significantly lower after 4 weeks' treatment with pramlintide compared with placebo (8.3 ± 0.7 *v* 10.2 ± 0.8 mmol/L, $P = .04$). However, preprandial plasma glucose did not differ in the two conditions either before breakfast (10.5 ± 1.1 *v* 11.5 ± 1.3 mmol/L, $P = .53$) or before lunch (8.0 ± 0.8 *v* 8.7 ± 1.1 mmol/L, $P = .57$). However, the postprandial (0 to 120 minutes) increase in plasma glucose as assessed by the incremental AUC was considerably lower during pramlintide administration at both meals (breakfast, −134 ± 56 *v* 125 ± 62 mmol/L · 120 min, $P = .001$; lunch, −117 ± 46 *v* 54 ± 70 mmol/L · 120 min, $P = .01$) (Fig 1).

As intended, the amount of insulin administered on the evening before the day of examination (18.0 ± 2.3 *v* 17.4 ± 2.3 U, pramlintide *v* placebo), before breakfast (9.0 ± 1.0 *v* 9.0 ± 1.0 U), and before lunch (6.1 ± 1.0 *v* 6.9 ± 1.0 U) was identical during the two regimens. Similarly, the total amount of insulin was comparable (33.1 ± 1.0 *v* 33.3 ± 3.2 U, pramlintide *v* placebo). In agreement with this, mean levels of circulating insulin during the day were comparable during the two conditions (359 ± 41 *v* 340 ± 35 pmol/L, P = NS, pramlintide *v* placebo) (Fig 1).

Plasma glucagon, serum GH, and leptin. Mean plasma glucagon levels during the daytime profile did not differ between pramlintide and placebo (49.9 ± 5.6 *v* 51.7 ± 6.6 ng/L), and analogously to plasma glucose, preprandial levels of circulating glucagon were comparable. The AUC for plasma glucagon during breakfast was diminished by 25% during pramlintide administration (5,707 ± 778 *v* 7,538 ± 902 ng/L · 120 min, pramlintide *v* placebo, $P = .02$), and a similar trend was observed following lunch, although not statistically

Table 2. Home Measurements of Blood Glucose

Study Week	Blood Glucose (mmol/L)		
	Pramlintide	Placebo	*P*
1*	8.5 ± 0.6	8.5 ± 0.4	.96
2	7.8 ± 0.6	7.7 ± 0.5	.87
3	7.9 ± 0.6	8.2 ± 0.5	.69
4	8.5 ± 0.6	8.5 ± 0.6	.92
Total period	8.3 ± 0.5	8.3 ± 0.4	.90

NOTE. Results are the mean of 4 measurements (breakfast, lunch, dinner, and bedtime).

*Mean of 3 successive days.

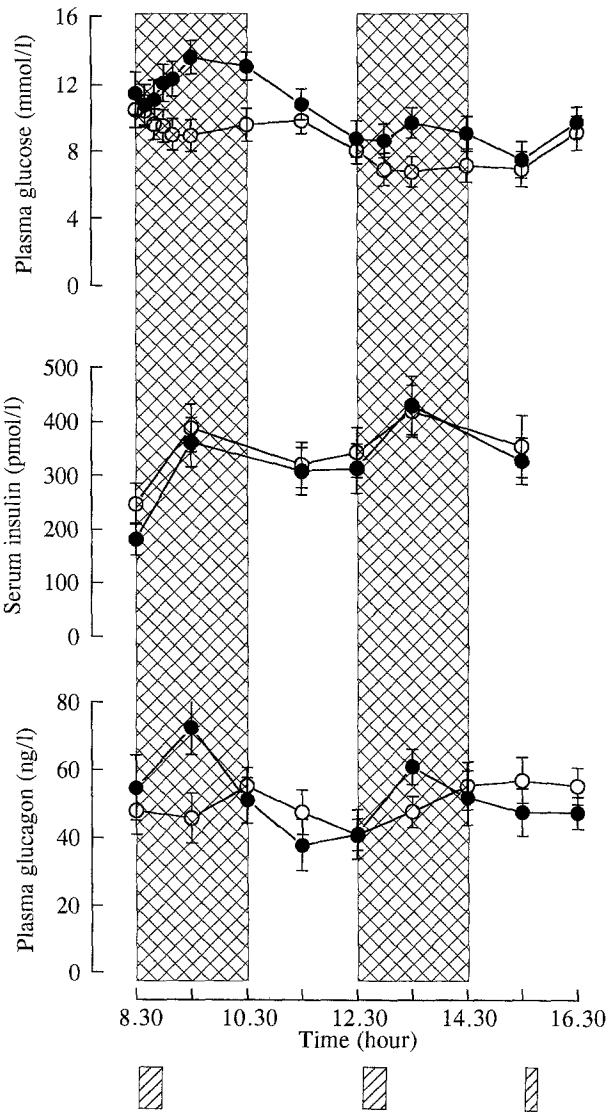

Fig 1. Plasma glucose, serum insulin, and plasma glucagon (mean ± SEM) during the daytime profile following pramlintide administration (○) and placebo (●). (▨) Postprandial periods following breakfast and lunch; (▨) meals.

significantly different (5,742 ± 835 *v* 6,441 ± 922 ng/L · 120 min, pramlintide *v* placebo). However, the incremental AUC for glucagon during the lunch period was reduced by 45% during pramlintide administration (831 ± 584 *v* 1,512 ± 373 ng/L · 120 min, pramlintide *v* placebo, $P = .06$). In addition, circulating glucagon concentrations 60 minutes after breakfast (45.8 ± 7.3 *v* 72.4 ± 8.0 ng/L, pramlintide *v* placebo, $P = .005$) and 60 minutes after lunch (47.6 ± 9.0 *v* 60.9 ± 8.2 ng/L, pramlintide *v* placebo, $P = .02$) were markedly diminished during pramlintide treatment (Fig 1).

Neither mean serum GH nor postprandial GH values differed in the two conditions (data not shown). Furthermore, circulating leptin concentrations measured before meals were not influenced by pramlintide treatment (breakfast, 2.04 ± 0.08 *v* 2.20 ± 0.16 ng/mL, $P = .18$; lunch, 1.95 ± 0.08 *v* 2.05 ± 0.09 ng/mL, $P = .24$, pramlintide *v* placebo).

Serum NEFA and blood metabolites. During pramlintide administration, mean blood glycerol levels were diminished (0.029 ± 0.004) as compared with placebo (0.040 ± 0.004 mmol/L, P ± .01). In parallel with this, serum NEFA and blood 3-OHB tended to decrease throughout the day during pramlintide administration, although not statistically significantly (0.264 ± 0.023 *v* 0.321 ± 0.028 mmol/L, P = .10, and 0.041 ± 0.008 *v* 0.086 ± 0.021 mmol/L, P = .07, respectively) (Fig 2).

Blood lactate profiles were almost superimposable during the two regimens (0.617 ± 0.038 and 0.645 ± 0.052 mmol/L, P = .84). In contrast, mean blood alanine concentrations were augmented after pramlintide treatment (0.274 ± 0.012 mmol/L) compared with placebo (0.246 ± 0.008 mmol/L, P = .03) (Fig 2).

As measured by a two-way ANOVA, no differences in the dynamic changes were observed in the daytime profiles for serum NEFA or blood metabolites during the two treatment regimens.

Adverse Events

When pramlintide was added to insulin treatment, 11 patients experienced 33 episodes of hypoglycemia, as compared with seven patients who experienced 13 episodes during placebo. The episodes, none of which were serious, were not always confirmed by blood glucose measurements. During the profile, three patients experienced hypoglycemia confirmed by plasma glucose measurements (defined as plasma glucose ≤ 2.8 mmol/L) during pramlintide treatment and were given an oral glucose supply. During placebo treatment, no patients developed hypoglycemia. Of the three episodes of hypoglycemia, one occurred in the morning and two in the afternoon. None occurred in the early (0 to 60 minutes) postprandial period. Some of the patients on pramlintide reported gastrointestinal adverse effects, primarily light abdominal discomfort (n = 1), anorexia (n = 3), vomiting (n = 1), and constipation (n = 1). Gastrointestinal symptoms were most pronounced at the beginning of the treatment period and tended to abate. Two patients had diarrhea during placebo administration. No patients withdrew from the study because of side effects or for any other reason. No serious or unexpected side effects were recorded.

DISCUSSION

The present study confirms data from recent reports demonstrating that short-term (weeks) pramlintide administration to type 1 diabetic individuals appears to influence glycemic control beneficially.[2-4] Although the study period was too short for HbA_{1c} to be a useful parameter, serum fructosamine, reflecting the glycemic level during the antecedent 3 to 4 weeks,[19] was 11% lower following pramlintide treatment. The reduction prevailed despite significantly lower 24-hour insulin requirements during the last 2 weeks of the 4-week treatment period. Diet records did not reveal differences in energy intake during the two treatment regimens. In concert with comparable preprandial home glucose measurements, the daytime profiles suggested that the improved glycemic control could be attributed mainly to a diminished postprandial glucose response. A decreased postprandial glycemic level following a Sustacal (Mead-Johnson Laboratories, Evansville, IN) meal challenge

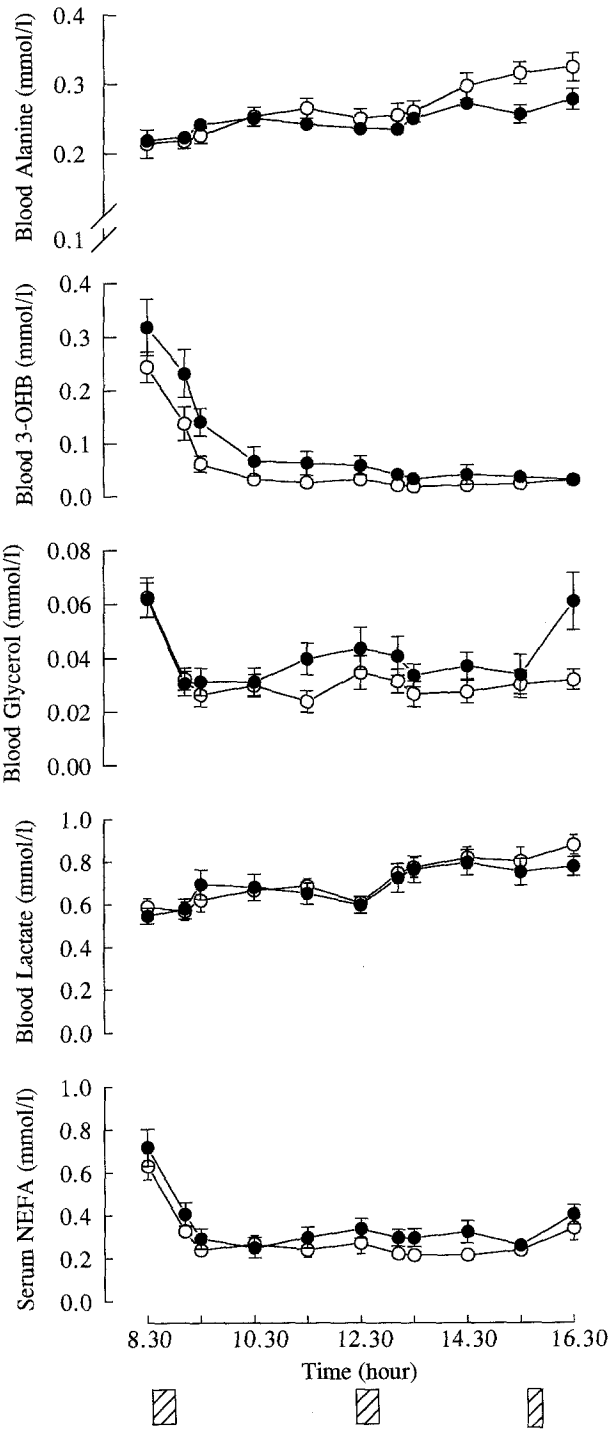

Fig 2. Blood intermediary metabolites and serum NEFA (mean ± SEM) during the daytime profile following pramlintide administration (○) and placebo (●). (▨) Meals.

has been demonstrated previously in type 1 diabetic patients after 2 weeks of pramlintide administration.[3,20] During the 4-week treatment period (pramlintide or placebo), patients were carefully monitored with respect to blood glucose control. One might therefore expect the insulin requirement to increase, which in fact was observed during placebo treatment. However,

during pramlintide treatment, the insulin dosage remained unaltered, indirectly indicating a diminished insulin requirement when insulin and pramlintide were both administered.

The mechanisms leading to improved glycemic control during pramlintide administration are not fully understood, although delayed gastric emptying (ie, slower delivery of dietary carbohydrates) is probably important. This action of amylin and amylin analogs, presumably mediated via the vagal nerve,[21] has been demonstrated in both rat and canine models[22,23] and recently also in type 1 diabetic individuals.[8] The present study suggests that another factor may contribute to the improved postprandial glycemic control. Diabetes mellitus is characterized by an inappropriately increased release of endogenous glucose,[24] especially in the postprandial period.[25] In addition, diabetes mellitus, even when well-controlled, is associated with glucagon excess both in the fasting and the postprandial state.[13] This glucagon hypersecretion is assumed to add to the glucose intolerance in insulin-deficient subjects[25,26] by stimulation of endogenous glucose output.

One of the novel observations in the present study is that pramlintide is capable of modifying glucagon levels in humans with type 1 diabetes mellitus, similar to the amylin-mediated suppression of arginine-induced glucagon secretion previously demonstrated in rats.[10] In our study, preprandial plasma glucagon concentrations were unaltered, but postprandial glucagon concentrations over a 2-hour period after the meals were diminished during pramlintide administration. Our finding is in line with recent data by Fineman et al,[27] who demonstrated that 2 weeks of pramlintide administration at a dosage of 30, 100, and 300 μg thrice daily reduced plasma glucagon levels in type 1 diabetic subjects following a Sustacal challenge. Although the differences in circulating (peripheral) glucagon concentrations may seem modest, they are rather dramatic when evaluated as a percentage. In this context, it is important to note that the acute effects of glucagon on endogenous glucose release are predominantly regulated by glucagon dynamics rather than absolute glucagon concentrations.[28-30] Furthermore, Dineen et al,[31] using the "pancreatic clamp technique," have recently shown that even small changes in postprandial glucagon concentrations affect endogenous glucose release significantly and aggravate postprandial hyperglycemia in individuals with type 1 diabetes mellitus.

The mechanism by which pramlintide modulates postprandial plasma glucagon concentrations is not clear. The failure of amylin to exhibit glucagonostatic effects in the isolated perfused rat pancreas[32] argues against a direct effect on the α cells. It is more plausible that the restraining effect of pramlintide on glucagon secretion is, at least in part, secondary to improved glycemic control[25] or, alternatively, to delayed gastric emptying, the latter leading to a more sluggish exposure of the protein nutrients capable of stimulating glucagon secretion. However, it may also be mediated through changes in other substrates, hormones, and peptides currently not well defined. Notably, Gedulin and Young[10] and Beumont et al[33] observed a glucagonostatic action of both amylin[10] and pramlintide[33] in response to an intravenous arginine challenge in rats, suggesting that amylin and pramlintide influence glucagon secretion independently of the effect on gastric emptying. Last but not least, the reduced glucagon secretion during pramlintide treatment could be mediated in part through the autonomic nervous system corresponding to the vagally mediated amylin effect on delayed gastric emptying.[21] Binding of amylin has been demonstrated in the area postrema in the hindbrain of the rat[34] involving the dorsal motor nucleus of the vagus. However, future studies are needed to delineate the modes of action of amylin and amylin analogs on glucagon secretion in more detail.

Many reports in rodents suggest that amylin may also be regarded as a satiety agent.[35] Although body weight tended to decrease more with pramlintide treatment, we were not able to detect differences in energy intake or body weight during the two 4-week regimens even though increased satiety was recorded in three subjects on pramlintide. To evaluate the possible role of another satiety hormone, leptin,[36] in mediating this effect, we determined fasting and pre-lunch leptin concentrations in blood. However, we failed to demonstrate differences between the two regimens, suggesting that the amylin-induced changes in satiety occur independently of leptin.

Another interesting observation in the current study is derived from the profiles of blood metabolites and serum NEFA. Serum NEFA, blood glycerol, and 3-OHB were or tended to be lower during pramlintide administration. This observation could be explained on the basis of improved glycemic control since the insulin concentrations were similar.[37] Whether the difference in glucagon dynamics during the two protocols might play a role is uncertain. It is established that glucagon in isolated adipose tissue possesses some lipolytic properties,[13] but it is controversial as to whether glucagon concentrations within the physiological range yield regulatory effects on fat metabolism in vivo.[38,39] However, the lower level of 3-OHB might reflect reduced hepatic ketogenesis, which could be secondary to the reduced glucagon levels.

In contrast to a previous study in rats[40] in which pharmacological doses of amylin led to an increased lactate level, blood lactate concentrations did not differ in this study. The discrepancy between the two studies may be grounded in differences between species and differences in the concentration of amylin or amylin analogs. We did not measure plasma concentrations of pramlintide in the present study, but according to a previous report using a similar pramlintide dosing regimen,[20] one would expect the levels to be within the range of postprandial amylin concentrations observed in lean healthy individuals. Blood alanine was significantly increased during pramlintide. Unpublished data from our laboratory did not indicate that pramlintide exposure modifies arteriovenous differences of alanine across the forearm (Ørskov L, Nyholm B, Hove KY, et al, 1996 to 1997). Therefore, it is tempting to suggest that the elevated alanine concentration is ascribable to reduced hepatic uptake of this gluconeogenic substrate, although investigations of the hepatic balance are needed to confirm this hypothesis.

Hypoglycemic episodes were almost threefold more frequent during pramlintide treatment compared with placebo. None of the episodes were serious, no episodes were reported to occur during the night, and preprandial blood glucose concentrations based on home glucose recordings were comparable during the two treatment periods. Thus, the increased number of hypoglycemic events during pramlintide administration occurred during the postprandial period. This apparent increase in hypoglycemic events contrasts with a previous report.[3] While the reason for

this discrepancy is not clear, the difference in fructosamine values observed between the two treatment regimens together with the established relationship between a significant improvement in glycemic control and the number of hypoglycemic events (eg, the Diabetes Control and Complications Trial[41]) make the observed differences in the incidence of hypoglycemic events somewhat predictable. In this regard, it should be noted that some of our patients exhibited good glycemic control at entry into the study, and the mean daily insulin dose was unaltered during the pramlintide period despite a significant improvement in glycemic control. Since all of our patients were on multiple-injection therapy, it is possible that alterations (decreases) in the ratio between soluble and intermediate-acting insulin reduced the number of hypoglycemic events recorded during pramlintide therapy.

In conclusion, 4 weeks' administration of the amylin analog pramlintide subcutaneously at a dose of 30 μg four times daily (prior to the three major meals and at bedtime) to type 1 diabetic individuals resulted in improved glycemic control as assessed by fructosamine, probably mainly due to reduced postprandial glucose excursions. A diminished circulating glucagon level might contribute to the latter. Future studies are needed to clarify the mechanism(s) by which pramlintide influences glucagon secretion and to what extent the improved glycemic control following pramlintide administration can be explained by this finding.

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