Effects of Pramlintide, an Analog of Human Amylin, on Plasma Glucose Profiles in Patients With IDDM

Results of a Multicenter Trial

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The effects of subcutaneous administration of 10, 30, or 100 μg q.i.d. pramlintide, an analog of human amylin, on plasma glucose regulation in patients with IDDM were evaluated in a multicenter trial. The plasma glucose response to a Sustacal test meal was significantly reduced compared with placebo both after 1 week and after 2 weeks of administration of 30 or 100 μg pramlintide. In addition, 24-h mean plasma glucose concentrations were significantly lowered in patients receiving 30 μg of pramlintide for 2 weeks compared with placebo, while the 100-μg pramlintide dose did not reach statistical significance for the 24-h glucose profiles. At 10 μg, pramlintide had no effect on the 24-h glucose profile or on the plasma glucose response to a Sustacal test meal. The reduction in 24-h glucose concentrations and glucose concentrations after the Sustacal test meal observed at the 30-μg pramlintide dose was not accompanied by an increased incidence of hypoglycemic events. The most frequent adverse events were dose-related and involved transient upper gastrointestinal symptoms. A majority (>80%) of the patients who reported these adverse events during week 1 did not report them in week 2. These data indicate that pramlintide effectively reduces plasma glucose concentrations as reflected in both a 24-h glucose profile and a Sustacal test meal while maintaining an acceptable safety profile. *Diabetes* 46:632–636, 1997

The polypeptide hormone amylin has been isolated and characterized as the major component of pancreatic amyloid deposits from patients with NIDDM (1). In normal human subjects, amylin concentrations vary in response to blood glucose concentrations. Fasting concentrations of amylin range between 4 and 11 pmol/l in normal subjects and increase two- to threefold following a mixed meal or during an oral glucose tolerance test (2–5). In patients with IDDM, amylin concentrations range from the lower end of the normal range to undetectable (6,7) and do not increase in response to a glucose load. Recent studies support the idea that supplementation with the human amylin analog pramlintide may lead to improved glucose regulation in patients with IDDM (8).

Human amylin, which is composed of 37 amino acid residues, aggregates ex vivo to form insoluble fibrils and is therefore not easy to formulate into a therapeutic agent. Pramlintide, an analog of human amylin, incorporates proline substitutions at positions 25, 28, and 29 of the amylin molecule. In this molecular form, the tendency of the peptide to aggregate is reduced while its desired biological activities are maintained. Pramlintide was therefore selected for further development.

Pramlintide was found in animal studies to display a spectrum of biological activity similar to that of endogenous amylin (9). Recent human studies with pramlintide administered three times a day for 14 days demonstrated a significant reduction in the postprandial glucose response after a Sustacal test meal (10). The present study was designed as a double-blind, randomized, placebo-controlled, parallel-group, fixed-dose study comparing the effects on 24-h plasma glucose profiles, pharmacokinetics, and drug tolerance of three doses of pramlintide and placebo administered four times a day for 14 days to patients with IDDM. This protocol also extended earlier findings by measuring responses to a standardized Sustacal test meal at baseline and at 7 and 14 days of pramlintide administration.

RESEARCH DESIGN AND METHODS

Patients. Study participants were selected by 16 investigators (see ACKNOWLEDGMENTS) from patients 18–60 years of age with a history of IDDM between 1.3 and 28.7 years' duration. HbA_{1c} levels were <13% (normal reference range for assay 4.30–6.10%), and basal C-peptide concentrations were <1.0 ng/ml (normal reference range for assay 0.5–3.0 ng/ml) in all patients except five who were enrolled because of a history of diabetic ketoacidosis. Patients were negative for serum hepatitis B surface antigen (HB_sAg) and had maintained stable body weight before admission into the study.

All procedures were conducted following approval of the duly constituted Institutional Review Board. At the screening visit, informed consent was obtained, and each patient underwent a physical examination and laboratory screening. Patients were instructed to remain on their usual diet, insulin, and exercise regimen throughout the study unless instructed otherwise by the investigator. Patients who met all criteria and successfully completed the screening visit reported to the clinic within 10 days to begin an initial 8-day, single-blind, placebo lead-in period. This was followed by a 2-week study drug period during which patients were randomized to one of four study drug groups, with subcutaneous self-administration of either placebo, 10 μg, 30 μg, or 100 μg q.i.d. pramlintide.

Patients self-monitored preprandial and bedtime blood glucose concentrations, daily insulin requirements, and hypoglycemic signs and symptoms along with dietary supplements (snacks) throughout the placebo lead-in and study drug periods. Adverse events observed by the investigator or volunteered by the patient were recorded at each visit.

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AUC, area under the curve; C_{max}, 24-h maximum glucose concentration; C_{min}, 24-h minimum glucose concentration; DCCT, Diabetes Control and Complications Trial.

TABLE 1
Demographic characteristics by study drug group

	Pramlintide q.i.d.			
	10 μg	30 μg	100 μg	Placebo q.i.d.
n	43	41	42	42
Age (years)				
Mean ± SE	36.6 ± 1.4	36.7 ± 1.5	37.0 ± 1.5	35.3 ± 1.5
Minimum–maximum	18–56	22–58	19–60	20–57
Height (inches)				
Mean ± SE	68.8 ± 0.6	68.7 ± 0.5	69.1 ± 0.5	70.1 ± 0.6
Minimum–maximum	57.0–76.0	62.0–74.0	60.0–74.0	63.0–84.0
Weight (pounds)				
Mean ± SE	172.3 ± 4.9	170.6 ± 4.5	173.2 ± 4.8	168.9 ± 3.1
Minimum–maximum	116–243	120–230	121–243	131.1–214
Duration of IDDM (years)				
Mean ± SE	11.49 ± 1.05	12.22 ± 1.11	12.50 ± 1.17	12.95 ± 1.10
Minimum–maximum	1.83–26.5	2.0–27.00	2.00–28.67	1.33–25.00
Sex				
Male	30 (69.8)	31 (75.6)	31 (73.8)	35 (83.3)
Female	13 (30.2)	10 (24.4)	11 (26.2)	7 (16.7)
Race				
White	40 (93.0)	36 (87.8)	39 (92.9)	40 (95.2)
Black	1 (2.3)	2 (4.9)	2 (4.8)	0 (0.0)
Hispanic	1 (2.3)	2 (4.9)	1 (2.4)	2 (4.8)
Asian	0 (0.0)	1 (2.4)	0 (0.0)	0 (0.0)
Middle Eastern	1 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)

Date for sex and race are *n* (%).

Placebo lead-in period. A 7-day placebo lead-in was included to assess the impact of participation in a clinical trial due to increased attention to compliance with diet and glucose monitoring. On the 1st day of the placebo lead-in period (study day 1), each patient received a standardized Sustacal test meal (Mead-Johnson Laboratories, Evansville, IN). The Sustacal test meal (7.0 kcal/kg) was consumed over a 15-min period. Patients administered their usual morning dose of insulin 30 min before the meal and their dose of placebo 15 min before the meal. Blood samples were taken every 30 min starting before the meal and for 3 h following the meal to determine plasma glucose and insulin concentrations.

On the 8th day of the placebo lead-in period (study day 8), patients entered the clinic for an overnight stay and measurement of the 24-h glucose profile. During this 24-h evaluation period, patients consumed a standard morning, noon, and evening meal followed by a snack at 9:00 P.M. Fifteen minutes before each meal and snack, a placebo injection was self-administered. Patients also received a mid-morning and mid-afternoon sugar-free beverage. Twenty-five blood samples were obtained throughout the 24-h period for measurement of plasma glucose and insulin concentrations. Twenty-four-hour mean glucose calculated as area under the curve (AUC) divided by the total collection time, 24-h maximum glucose concentration (C_{max}), and 24-h minimum glucose concentration (C_{min}) were evaluated. The results for this 24-h evaluation period served as a baseline for the study drug phase of the trial.

After completion of the 24-h profile, the Sustacal meal test was repeated (study day 9) as described for study day 1. The results for this Sustacal meal test served as a baseline for the study drug phase of the trial. Following the 3-h postmeal evaluation period, patients were discharged from the clinic after randomization to one of the four study drug groups.

Study drug period. After discharge from the clinic on study day 9, patients resumed their usual diet, insulin, and exercise regimen and began the study drug period of the trial, which included preprandial administration of placebo, 10 μg, 30 μg, or 100 μg pramlintide. On the morning of the 7th day of randomized study drug administration (study day 15), patients reported to the clinic for a Sustacal meal test conducted with the same protocol as used on study day 1.

After 14 days of study drug administration (study day 22), patients entered the clinic for a second 24-h plasma glucose profile. The Sustacal meal test was repeated the following morning (study day 23).

As an assessment of study drug response, patients were classified as responders if their mean glucose was reduced by at least 0.8 mmol/l during the final 24-h glucose profile compared with baseline.

Measurements

Plasma glucose concentrations. Blood samples were immediately centrifuged and the plasma was separated, transferred to a clean tube, and stored frozen until analysis. Plasma glucose concentrations were measured by Endocrine Sciences (Calbasas Hills, CA) using the glucose hexokinase method.

Blood HbA_{1c} levels. Whole blood samples containing EDTA were hemolyzed for the HbA_{1c} assay. HbA_{1c} levels were measured by Corning SciCor Laboratories (Indianapolis, IN) using a BioRad Variant high-performance liquid chromatography (HPLC) system with a detection limit of 3.6% and a normal range of 4.30–6.10% for nondiabetic subjects.

Serum C-peptide concentrations. Samples of venous blood were collected in serum separator tubes and allowed to clot for 30 min for measurements of serum C-peptide concentrations along with other blood chemistry parameters. Serum C-peptide concentrations were measured by Corning SciCor Laboratories using the INCStar radioimmunoassay kit, with a detection limit of 0.1 ng/ml and a normal range of 0.5–3.0 ng/ml.

Statistical analysis. For a patient to be eligible for evaluation for the 24-h plasma glucose profile, there must have been no more than three missing glucose values in either 24-h profile. For a patient to be eligible for evaluation for the Sustacal meal test, there must have been no more than one missing glucose value in any of the Sustacal meal tests.

Two-way analysis of variance with the Hochberg adjustment (11) for multiple comparisons was performed on continuous variables. Due to the non-normality assumptions not being met, the Sustacal meal test data were analyzed using nonparametric procedures. For nonparametric analyses, a Wilcoxon rank-sum test with the Hochberg adjustment for multiple comparisons was performed. For categorical variables, a Cochran-Mantel-Haenszel analysis with the Hochberg adjustment for multiple comparisons was performed. Results compared to baseline after 1 and 2 weeks of study drug administration were calculated by subtracting the baseline results from the 1- and 2-week results, respectively. For these calculations, parameters that decreased after 1 and 2 weeks were expressed as negative values, and parameters that increased were expressed as positive values.

RESULTS

Patient disposition. Of the 168 patients randomized,165 completed the study. One hundred fifty-nine patients were evaluable for the 24-h plasma glucose profile, and 145 patients were evaluable for the Sustacal meal test.

TABLE 2
Baseline HbA_{1c} and C-peptide

Study drug group	*n*	%	*P* value
HbA_{1c}			
Placebo q.i.d.	39	8.6 ± 0.2	—
Pramlintide 10 μg	40	8.7 ± 0.3	NS
Pramlintide 30 μg q.i.d.	40	8.8 ± 0.2	NS
Pramlintide 100 μg q.i.d.	40	9.3 ± 0.2	NS
C-peptide			
Placebo q.i.d.	39	0.45 ± 0.04	—
Pramlintide 10 μg q.i.d.	39	0.37 ± 0.03	NS
Pramlintide 30 μg q.i.d.	40	0.48 ± 0.04	NS
Pramlintide 100 μg q.i.d.	39	0.42 ± 0.03	NS

Data are means ± SE for evaluable patients for the 24-h glucose profile. *P* value calculated by two-way analysis of variance with the Hochberg adjustment.

Demographic characteristics. The demographic characteristics of the four study drug groups were similar, as shown in Table 1.

Baseline comparability of the study drug groups. Overall, the four study drug groups were comparable with regard to HbA_{1c} and C-peptide levels upon admission into the study, as shown in Table 2. The correlation between baseline HbA_{1c} and baseline mean 24-h plasma glucose concentration was positive for the placebo, 10-μg pramlintide, and 30-μg pramlintide groups (r = 0.36, 0.33, and 0.46, respectively) but was poor for the 100-μg pramlintide group (r = –0.02).

Sustacal meal test. Plasma glucose AUC following the Sustacal test meal after 1 and 2 weeks of study drug administration was compared with plasma glucose AUC at the end of the placebo lead-in phase of the study. There was a statistically significant median reduction in glucose AUC compared with placebo following 1 and 2 weeks of administration of 30 μg pramlintide q.i.d. (755.6 and 247.7 mmol · min^{-1} · l^{-1}, respectively) or 100 μg pramlintide q.i.d. (650.3 and 303.1 mmol · min^{-1} · l^{-1}, respectively). In addition, the median reduction in glucose AUC after 1 week of pramlintide administration was statistically significantly greater in the 30-μg and 100-μg groups than in the 10-μg group (P = 0.0035 and 0.0034, respectively). Following 1 and 2 weeks of 10 μg pramlintide, the median reduction in glucose AUC (12.9 and 149.0 mmol · min^{-1} · l^{-1}, respectively) was not statistically significant compared with placebo (22.1 mmol · min^{-1} · l^{-1} reduction and 172.8 mmol · min^{-1} · l^{-1} increase in AUC, respectively).

Twenty-four-hour plasma glucose profile. Mean 24-h plasma glucose concentrations after the placebo lead-in period and after 2 weeks of placebo or pramlintide are shown in Fig. 1. Patients in the 30-μg pramlintide group had a statistically significant reduction in mean glucose (1.9 ± 0.4 mmol/l) compared with patients in the placebo group (0.03 ± 0.5 mmol/l). The reductions in mean glucose for the 10- and 100-μg pramlintide groups did not reach statistical significance compared with placebo. There were no statistically

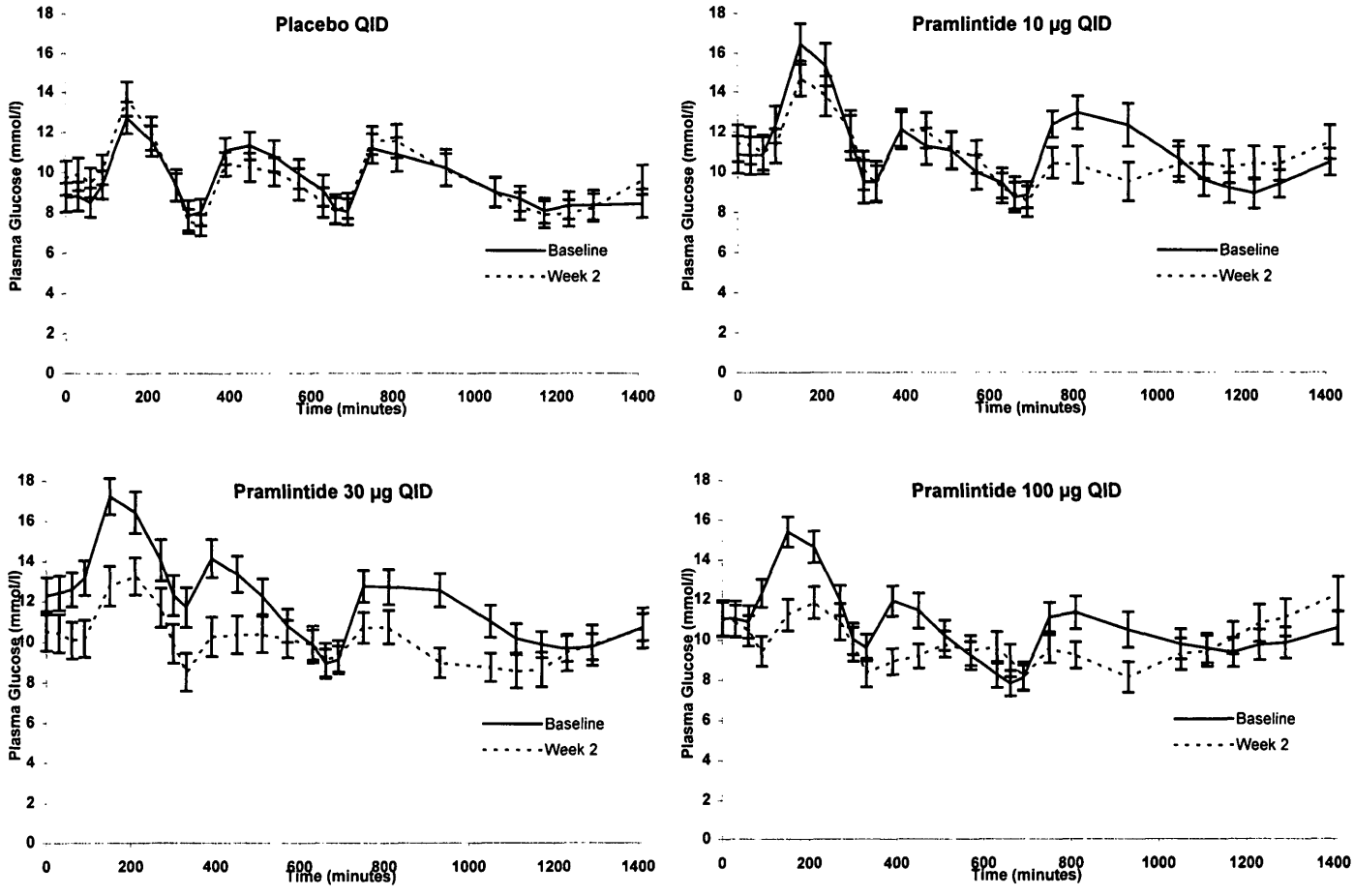

FIG. 1. Plasma glucose (mean ± SE) concentrations during 24-h test periods after placebo lead-in and after 2 weeks of pramlintide or placebo administration for the four study groups.

TABLE 3
Responder analysis based on reduction in 24-h mean glucose

Study drug group	n	0.8 mmol/l responders n	%	P value
Placebo q.i.d.	39	14	35.9	—
Pramlintide 10 µg q.i.d.	40	17	42.5	NS
Pramlintide 30 µg q.i.d.	40	28	70.0	0.003
Pramlintide 100 µg q.i.d.	40	16	40.0	NS

A responder was defined as a patient with at least a 0.8 mmol/l reduction in mean glucose from baseline. P value was calculated using the Cochran-Mantel-Haenszel test with the Hochberg adjustment.

significant reductions in glucose C_{max} or glucose C_{min} compared with placebo in any pramlintide group. The reduction in glucose C_{min} was also statistically significant in the 30-µg pramlintide q.i.d. group (1.5 ± 0.4 mmol/l) compared with the 10-µg pramlintide q.i.d. group (0.05 ± 0.3 mmol/l) (P = 0.0066), but not compared with the reduction in glucose C_{min} in the placebo group (0.2 ± 0.3 mmol/l).

The number of patients in each study drug group classified as responders is summarized in Table 3. Responders had no noticeable differences from nonresponders based on demographic characteristics or baseline HbA_{1c} levels.

The occurrence of glucose concentrations <2.8 mmol/l during the 24-h profile revealed no change in the percentage of patients in the placebo group with at least one glucose concentration <2.8 mmol/l at baseline (33.3%) and after 2 weeks of placebo administration (35.9%). At baseline, the percentage of patients with at least one glucose concentration <2.8 mmol/l was similar in the 10-µg pramlintide group and the placebo group, but the percentage was lower in the 30-µg pramlintide (17.5%) and 100-µg pramlintide (17.5%) groups. After 2 weeks of study drug administration, the percentage in the latter two groups increased (35.0%, 30 µg pramlintide; 42.5%, 100 µg pramlintide) and was similar to the percentage of patients in the placebo (35.9%) and 10-µg pramlintide groups (35.0%) after 2 weeks of study drug.

Even though patients and investigators were allowed to alter insulin doses based on home glucose monitoring results, there were no consistent differences in these changes between groups, and no statistically significant differences in plasma insulin concentrations were observed between the four study drug groups.

Safety data. The most frequent adverse events involved upper gastrointestinal symptoms (nausea, anorexia, and dyspepsia) and occurred more frequently in patients in the pramlintide groups than in patients in the placebo group. In the pramlintide groups, there appeared to be a dose-response relationship for nausea and anorexia. Nausea was reported in 2.4% of patients in the placebo group and 2.3, 19.5, and 42.9% of patients in the 10-, 30-, and 100-µg pramlintide groups, respectively. Anorexia was reported by no patients in the placebo group and by 0.0, 2.4, and 9.5% of patients who received 10, 30, and 100 µg q.i.d., respectively. No patients on pramlintide who reported anorexia in the 1st week reported this adverse event in the 2nd week of administration. Nausea was no longer a problem in week 2 for >80% of the patients who reported nausea in week 1.

Hypoglycemia was frequently reported by patients in all phases of the study. More than 75% of the patients enrolled in the trial reported hypoglycemia during the placebo lead-in period. The incidence during the 2-week study period was 34 of 43 patients (79.1%) in the 10-µg group, 35 of 41 patients (85.4%) in the 30-µg group, 34 of 42 patients (81.0%) in the 100-µg group, and 34 of 42 patients (81.0%) in the placebo group.

DISCUSSION

Pramlintide at 30 µg q.i.d. resulted in a statistically significant reduction in 24-h mean plasma glucose compared with placebo in patients with IDDM. Pramlintide at 100 µg showed a similar trend that failed to achieve statistical significance (P = 0.055). It is of note that the correlation between the baseline 24-h glucose and the HbA_{1c} for the 100-µg group suggests that the baseline 24-h glucose was lower than anticipated from the HbA_{1c}. It should also be noted that plasma glucose concentrations, as measured by 24-h glucose AUC, were higher in the 30-µg pramlintide group than in the placebo group at baseline. After 2 weeks of administration, the plasma glucose AUC values were similar between the 30-µg pramlintide group and the placebo group. As each group served as its own control, the statistical design of the study was able to control for these differences in baseline measurements. In addition to the reduction in 24-h plasma glucose concentrations in the 30-µg pramlintide group, the responder analysis demonstrated that a statistically significantly greater percentage of patients responded to study drug administration with a reduction of 0.8 mmol/l of plasma glucose or greater in the 30-µg pramlintide group compared with the placebo group. Therefore, the reduction in plasma glucose by 30 µg of pramlintide in patients with IDDM was both statistically and clinically relevant.

Mean 24-h glucose concentrations have been shown to correlate with HbA_{1c} concentrations in patients with IDDM (12). Although the current study was too brief to directly measure a decrease in HbA_{1c}, a statistically significant decrease of 1.9 ± 0.4 mmol/l in mean 24-h plasma glucose concentration was observed with 30 µg pramlintide in this 2-week study. A decrease of this magnitude would be expected to result in a clinically relevant decrease in HbA_{1c} if these glucose reductions were maintained over a longer time.

The number of patients with plasma glucose concentrations less <2.8 mmol/l during the 24-h plasma glucose profile increased over the 2-week study drug period in the 30- and 100-µg pramlintide groups. However, there was no apparent relationship between the dose of pramlintide administered and glucose concentrations <2.8 mmol/l. Moreover, the incidence of hypoglycemic events was no higher in the 30- and 100-µg pramlintide groups than in the placebo group during the 2 weeks of study drug administration. Although glucose C_{min} decreased in the 30-µg pramlintide group, this reduction was not statistically significantly different from that in the placebo group and did not appear to correspond to an increased incidence of hypoglycemic events. While studies of longer duration are required for confirmation, these findings suggest that pramlintide reduces 24-h plasma glucose concentrations by a mechanism that may provide a desirable margin of safety against hypoglycemia that is difficult to achieve with traditional insulin therapy alone.

The incidence of hypoglycemia is based on self-reports by the patient that were not confirmed by concurrent blood glu-

cose assessments in many cases. Thus, these reports of mild and moderate hypoglycemia may include a number of symptomatic episodes that did not represent hypoglycemia. The ethical need to disclose hypoglycemia as a potential side effect of pramlintide is more important than over-reporting these symptoms.

The effectiveness of intensive insulin therapy in delaying the development and progression of diabetic retinopathy, nephropathy, and neuropathy in patients with IDDM was demonstrated in the Diabetes Control and Complications Trial (DCCT) (13). The improved glycemic control observed in the DCCT was associated with an increase of approximately threefold in the incidence of severe hypoglycemia. The difficulties associated with intensive insulin therapy, including an increase in severe hypoglycemia and a 73% higher risk of becoming overweight, were discussed extensively in an additional publication from the same research group (14). The DCCT Research Group concluded in the discussion of adverse events that efforts must be focused on refining established interventions and developing new interventions designed to minimize the sustained increased risk of severe hypoglycemia involved in intensive treatment with insulin. The observation that addition of pramlintide to the usual insulin regimen of patients with IDDM resulted in a significant and clinically relevant reduction in mean 24-h plasma glucose concentrations without an apparent increase in hypoglycemic events points to a means of potentially achieving improved glucose control without added risk.

The mechanism leading to the reduction in 24-h glucose concentrations by pramlintide at the 30-μg dose is not fully defined, although modulation of gastric emptying appears to explain at least part of the results. In a previous study (8), intravenous infusion of pramlintide decreased postprandial plasma glucose concentrations in patients with IDDM after a Sustacal meal challenge but not after an intravenous glucose load. A recent study showed that intravenous infusions of pramlintide modulated gastric emptying of both liquids and solids in patients with IDDM (15). This action of pramlintide could result in slowed glucose absorption, thereby reducing the usual increase in postprandial plasma glucose concentrations. This mechanism of action is also supported by reports demonstrating that gastric emptying is slowed by amylin administration in both dogs (16) and rats (17).

In summary, pramlintide lowered 24-h mean glucose concentration in patients with IDDM and improved postprandial glucose control following a Sustacal test meal. These effects were demonstrated without compromising safety; the doses of pramlintide tested were generally well tolerated during the 1st week of administration and tolerability improved after the 1st week. In this 2-week study, the 10-μg dose of pramlintide was below the minimum effective dose. The smaller effects of 100 μg pramlintide than 30 μg of pramlintide on glucose concentrations during the 24-h profile may reflect a change in the patients' behavior during the baseline glucose profile. Future studies are needed using measures of glycemic control (fructosamine and HbA_{1c}) that are not influenced by patient behavior at the time the blood is drawn (glucose profile) to further explore the effects of pramlintide on glycemic control in patients with IDDM.

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