The Human Amylin Analog, Pramlintide, Corrects Postprandial Hyperglucagonemia in Patients With Type 1 Diabetes

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Mealtime amylin replacement with the human amylin analog pramlintide as an adjunct to insulin therapy improves postprandial glycemia and long-term glycemic control in type 1 diabetes. Preclinical animal studies indicate that these complementary effects may result from at least 2 independent mechanisms: a slowing of nutrient delivery to the small intestine and a suppression of nutrient-stimulated glucagon secretion. The former effect of pramlintide has previously been demonstrated in patients with type 1 diabetes. The present studies characterize the effect of pramlintide on postprandial glucagon secretion in this patient population. Plasma glucagon and glucose concentrations were measured before and after a standardized liquid meal in 2 separate randomized, double-blind, placebo-controlled studies of pramlintide administration to patients with type 1 diabetes. In a 2-day crossover study, 18 patients received a 5-hour intravenous infusion of pramlintide (25 μg/h or 50 μg/h) or placebo in addition to subcutaneous (SC) insulin injections. In a 14-day parallel-group study, 84 patients received SC injections of 30, 100, or 300 μg of pramlintide or placebo 3 times daily in addition to SC injections of insulin. In both studies plasma glucagon concentrations increased in response to the meal in the placebo-plus-insulin group but not in any of the pramlintide-treated groups (all pramlintide treatment arms *v* placebo, $P < .05$). We conclude that mealtime amylin replacement with pramlintide prevents the abnormal meal-related rise in glucagonemia in insulin-treated patients with type 1 diabetes, an effect that likely contributes to its ability to improve postprandial glucose homeostasis and long-term glycemic control.

AMYLIN IS A 37–amino acid polypeptide hormone that is colocalized with insulin in β-cell secretory granules and is cosecreted with insulin in response to nutrient stimuli.[1,2] Preclinical studies performed in rodents and primates have shown that amylin acts as a neuroendocrine hormone that binds with high affinity to selective regions of the brain, including the *area postrema*, and elicits several effects that collectively regulate the rate of glucose influx into the circulation after meals.[3,4] Amylin's effects are therefore complementary to the effects of insulin, which is the primary hormonal regulator of glucose efflux.[3,4]

In type 1 diabetes, β cells are destroyed by an autoimmune process, thus rendering patients deficient in both insulin and amylin.[4,5] Randomized, double-blind, placebo-controlled clinical studies have shown that mealtime amylin replacement via subcutaneous (SC) injection of the synthetic human amylin analog, pramlintide, as an adjunct to insulin therapy, improves postprandial glucose excursions and long-term glycemic control (HbA_{1c}) in patients with type 1 diabetes.[6-8]

One well-documented mechanism for the postprandial glucose-lowering effect of pramlintide is its effect on gastric emptying. Preclinical animal studies and studies in patients with type 1 diabetes have demonstrated that pramlintide slows the delivery of nutrients from the stomach to the small intestine to a rate that better matches the rate of glucose disposal,[9,10] resulting in improved postprandial glucose control. Experimental data indicate that as much as 35% of the variance in peak postprandial glucose excursions in both healthy subjects and patients with type 1 diabetes is attributable to variations in the rate of gastric emptying.[11]

A second, independent, effect of amylin identified in animal studies is a potent dose-dependent suppression of nutrient-stimulated glucagon secretion.[12] By suppressing glucagon, the major hormonal stimulator of hepatic glucose output, amylin appears to also regulate the influx of liver-derived (endogenous) glucose into the circulation, thereby further contributing to postprandial glucose control. Inappropriate hypersecretion of glucagon and an inability to adequately suppress hepatic glucose output at mealtime are well documented in patients with type 1 diabetes and both abnormalities are considered to be important contributors to unsatisfactory postprandial glycemic control in these patients.[13,14]

The aim of the present series of studies was to examine the effect of pramlintide on postprandial glucagon secretion in patients with type 1 diabetes.

MATERIALS AND METHODS

We measured plasma glucagon and glucose concentrations in 2 separate, randomized, double-blind, placebo-controlled studies, involving either a single intravenous infusion or 14-day SC injection of pramlintide (Amylin Pharmaceuticals, Inc, San Diego, CA). Patients in both studies were 18 to 51 years of age, with a history of type 1 diabetes as defined by National Diabetes Data Group Criteria,[15] basal plasma C-peptide concentrations of less than 1.0 ng/mL, disease duration of 2 to 26 years, and HbA_{1c} values of less than 14%. The respective institutional review boards approved the protocols and all volunteers completed approved informed consent documents prior to initiating study procedures.

Study 1 (Single Intravenous Infusion)

Study 1 utilized a 2-day crossover design to examine the effect of a single continuous (5-hour) intravenous infusion of pramlintide on glucagon secretion before and after a standardized meal in 16 patients with type 1 diabetes. Patients were divided into 2 groups. In randomized order, one group of patients (n = 9) received either a placebo infusion or a pramlintide infusion at a rate of 25 μg/h on 2 consecutive days. Again, in randomized order, the second group (n = 9) received either

From Amylin Pharmaceuticals, San Diego, CA.

Submitted August 7, 2001; accepted November 19, 2001.

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0026-0495/02/5105-0018$35.00/0

doi:10.1053/meta.2002.32022

Table 1. Demographics and Baseline Characteristics

	Study 1: Acute Intravenous Infusion (dose)		Study 2: Chronic SC Administration (dose)			
	25 μg/h	50 μg/h	Placebo	30 μg	100 μg	300 μg
No. of patients	9	9	22	18	23	21
Gender (M/F)	8/1	7/2	14/8	11/7	19/4	19/2
Race						
Caucasian	9	9	18	16	18	19
Asian			0	0	1	0
Black			2	1	1	1
Hispanic			2	1	3	1
Age* (yr)	28.4 ± 3.6 (19-36)	32.6 ± 2.7 (24-40)	37.0 ± 1.8 (21-48)	35.5 ± 1.8 (23-46)	33.0 ± 1.8 (18-49)	33.2 ± 2.2 (18-51)
BMI* (kg/m^2)	22.7 ± 0.4 (22.0-23.8)	24.2 ± 1.4 (20.2-27.0)	24.7 ± 0.6 (18.7-28.5)	23.7 ± 0.6 (19.6-29.3)	24.2 ± 0.6 (19.5-29.3)	24.7 ± 0.6 (18.2-29.2)
Disease duration* (yr)	6.9 ± 2.0 (2-12)	10.8 ± 2.7 (3-19)	11.4 ± 1.6 (2-26)	10.4 ± 1.7 (3-31)	9.2 ± 1.1 (1-23)	13.8 ± 1.4 (3-24)
Age at onset* (yr)	21.5 ± 4.8 (8-30)	21.8 ± 3.1 (13-32)	25.6 ± 2.0 (6-40)	25.1 ± 2.3 (10-42)	23.9 ± 2.0 (7-45)	19.4 ± 2.5 (5-41)
Baseline HbA_{1c}* (%)	9.4 ± 1.0 (7.0-11.5)	9.3 ± 0.6 (7.5-11.2)	8.9 ± 0.4 (6.6-13.7)	8.3 ± 0.4 (6.0-11.9)	8.8 ± 0.3 (6.4-12.4)	8.6 ± 0.4 (5.4-12.0)
Baseline insulin use* units,	29.1 ± 7.6	37.8 ± 17.3	51.4 ± 5.6	45.3 ± 5.4	49.3 ± 3.1	56.4 ± 2.8

*Values are mean ± SEM (range).

placebo or pramlintide infused at a rate of 50 μg/h on 2 consecutive days. In all cases, patients arrived at the clinic on the day prior to the test to become acclimated overnight. On the next day, 2 intravenous catheters were inserted, and the infusion of pramlintide or placebo was initiated at time (T) = 0 minutes. At T = 30 minutes, patients received a single SC injection of regular insulin dosed individually to cover the subsequent meal. At T = 60 minutes, patients ingested a standardized liquid meal (Sustacal, Mead Johnson, Evansville, IN, 355 kcal/355 mL, 55% carbohydrate, 21% fat, 24% protein). Blood samples were taken throughout the infusion period for measurement of plasma glucose, glucagon, and pramlintide concentrations. On the following day, the study protocol was repeated with the infusion of the second treatment arm (placebo or pramlintide), using the same Sustacal meal and the same dose of insulin.

Study 2 (14-Day SC Administration)

Study 2 used a parallel-group design to examine the effect of SC injections of various doses of pramlintide 3 times daily over 14 days on glucagonemia before and after a standardized meal in 84 patients with type 1 diabetes. Patients were randomized to receive SC injections of placebo or 30, 100, or 300 μg of pramlintide 3 times daily 30 minutes prior to each meal at a separate site from their usual insulin injection. Patients were instructed to maintain their usual insulin, diet, and exercise regimens throughout the study. After 14 days, patients returned to the clinic the morning after an overnight (10-hour) fast. After the insertion of an intravenous catheter for blood sampling, patients were given their usual morning insulin doses as well as their doses of pramlintide by separate injections. At T = 30 minutes, patients ingested a standardized liquid meal (Sustacal, 355 kcal/355 mL). Blood samples for determination of glucose, glucagon, and pramlintide were drawn for 3 hours starting at the point patients received insulin and pramlintide or placebo.

Analytical Methods

Plasma glucose concentrations were determined using the glucose oxidase method.[16] Plasma pramlintide concentrations were determined using an immunoradiometric assay (interassay coefficient of variation [CV] < 15%, intra-assay CV < 10%) as described previously.[10] Plasma glucagon concentrations were determined using a commercial radioimmunoassay kit (CV < 10%; Linco Research Inc, St Charles, MO).

Statistical Analysis

All graphical data are represented as mean ± SEM. Areas under the curve (AUCs) were calculated employing the trapezoidal rule. Comparison of means was performed using paired analysis of variance (ANOVA) and *t* tests or Dunnett's multiple comparisons test. Statistical analyses were performed using SAS version 6.11 (SAS Institute Inc, Cary, NC) and InStat version V2.04a (GraphPad, San Diego, CA).

RESULTS

The demographic and baseline characteristics of the patients from both studies are shown in Table 1. There were no group differences in age, weight, or duration of disease in either study.

Study 1 (Single Intravenous Infusion)

The mean changes from baseline in plasma glucagon concentrations are shown in Figs 1A and C. Collectively, patients receiving placebo injections together with their insulin injections, in both studies, had significant increases in plasma glucagon concentrations (17.9 ± 4.0 pg/mL; $P \leq .001$) in response to the meal. In contrast, in patients receiving pramlintide, 25 or 50 μg/h, the mean changes from baseline in glucagon concentrations, were unchanged or slightly suppressed (-3.9 ± 3.8 pg/mL and -11.5 ± 3.3 pg/mL, respectively; $P \leq .014$). Total glucagon secretion was also significantly decreased for both groups during pramlintide infusion, as reflected by the plasma glucagon AUC from the time of caloric challenge to the end of the infusion period (60 to 300 minutes). The glucagon AUC was 15,994 ± 1,295 pg · min/mL for placebo compared to

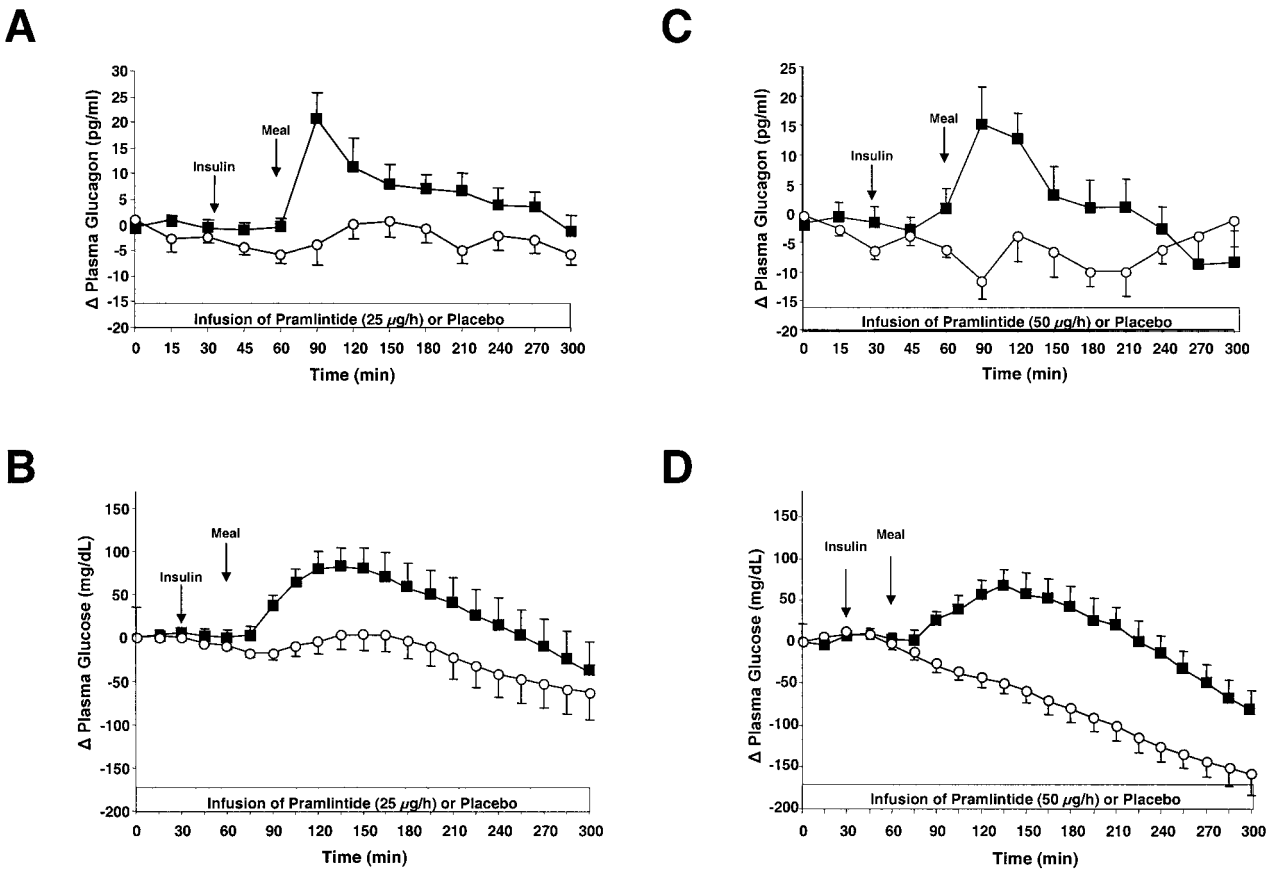

Fig 1. Study 1: Effects of intravenous pramlintide *v* placebo infusion on plasma glucagon and glucose concentrations. (A and B) Pramlintide (○) at 25 μg/h, placebo (■). (C and D) Pramlintide (○) at 50 μg/h, placebo (■). All data means ± SEM.

14,873 ± 2,518 pg · min/mL and 12,956 ± 72.6 pg · min/mL for the 25- and 50-μg/h pramlintide treatments ($P < .001$, ANOVA).

Fasting plasma glucose concentrations of placebo and treatment groups (determined prior to the test meal) were not significantly different between the 2 study days; the 25-μg/h group had fasting glucose concentrations of 251 ± 35 mg/dL (placebo) and 223 ± 19 mg/dL (pramlintide) and the 50-μg/h group had fasting glucose concentrations of 281 ± 22 mg/dL (placebo) and 273 ± 23 mg/dL (pramlintide) (mean ± SEM). The glycemic response to the meal was reduced in both pramlintide treatment groups compared to placebo as assessed by the glucose AUC above the fasting values (incremental glucose AUC) (Fig 1B and D). The mean ± SEM incremental glucose AUC for the 25-μg/h group was 6,954 ± 6,295 mg · dL^{-1} · min for pramlintide recipients versus 7,923 ± 7,941 mg · dL^{-1} · min for placebo recipients ($P \leq .001$). The 50-μg/h group had similar reductions in postprandial glucose concentrations for pramlintide recipients compared to placebo recipients (-27,652 ± 4,603 mg · dL^{-1} · min pramlintide *v* -769 ± 6,293 mg · dL^{-1} · min placebo; $P \leq .005$).

Mean steady-state plasma pramlintide concentrations (60 to 300 minutes) on the treatment days were 116 ± 7.5 pmol/L and 227 ± 23 pmol/L for the 25-μg/h and 50-μg/h dose groups.

Study 2 (14-Day SC Administration)

Figure 2A displays the mean changes in plasma glucagon concentrations from baseline. In the placebo group plasma glucagon rose in response to the meal by +17.0 ± 3.2 pg/mL at 30 minutes after the meal. This increase was blunted for all 3 pramlintide groups with negligible changes in postprandial plasma glucagon concentrations throughout the test (at 60 minutes: -1.5 ± 2.0, -1.2 ± 1.6, and 0.1 ± 2.6 for the 30-, 100-, and 300-μg doses, respectively, compared to placebo, $P < .001$). The total plasma glucagon response to the caloric challenge was also reduced in all 3 dose groups compared to placebo ($P < .05$). The mean plasma glucagon AUC adjusted for the fasting period (0 through 30 minutes) was 1,387 ± 275 pg · min/mL for placebo compared to 238 ± 125 pg · min/mL, 576 ± 261 pg · min/mL, and 48 ± 17 pg · min/mL for the 30-, 100-, and 300-μg/mL groups, respectively.

Fasting glucose concentrations among the 4 dose groups were similar: 196 ± 19 mg/dL (placebo), 156 ± 19 mg/dL (30 μg pramlintide), 194 ± 19 (100 μg pramlintide), and 193 ± 26 (300 μg pramlintide). However, by 30 minutes after the meal, the placebo group showed a significantly greater rise in plasma glucose concentration compared to the 3 pramlintide dose groups (Fig 2B). The AUCs for the treatment groups relative to

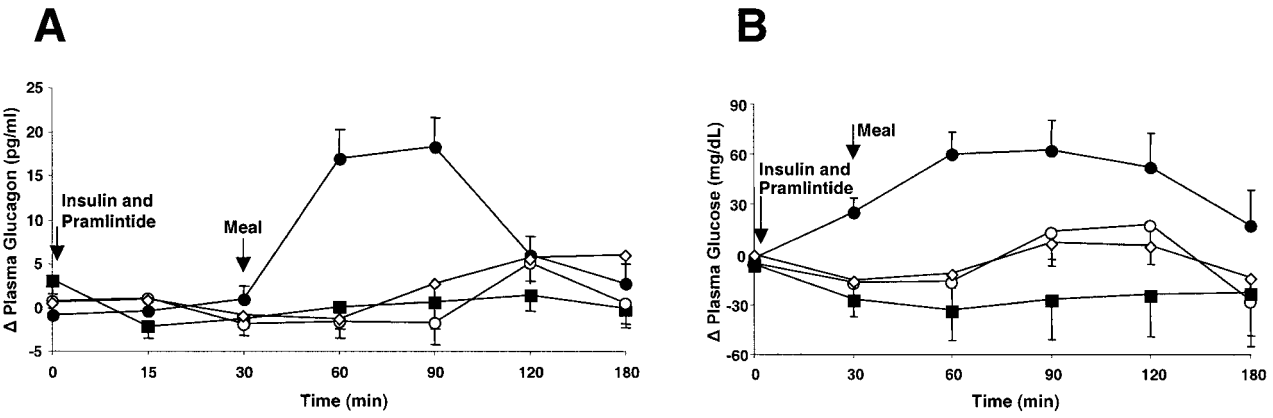

Fig 2. Study 2. (A and B) Effects of chronic pramlintide *v* placebo administration over 14 days on plasma glucagon and glucose concentrations. Pramlintide at (○) 30 μg, (◇) 100 μg, (■) 300 μg, and (●) placebo. All data are means ± SEM.

placebo for the 0- to 180-minute time period were 409.0 ± 2,477.9 mg · dL^{-1} · min for the 30-μg group, $P = .0269$; -727.8 ± 2,046.1 mg · dL^{-1} · min for the 100-μg group, $P = .0051$; and -3,403.8 ± 2,770.4 mg · dL^{-1} · min for the 300-μg group, $P = .0020$, compared to an AUC for placebo of 7,750.6 ± 2,094.2 mg · dL^{-1} · min.

Mean peak plasma pramlintide concentrations were 25.6 ± 4.5 pmol/L, 52.4 ± 14.6 pmol/L, and 192.8 ± 36.8 pmol/L for the 30-, 100-, and 300-μg dose groups, respectively.

DISCUSSION

Inappropriately high plasma glucagon concentrations and impaired suppression of hepatic glucose output at mealtime are considered to be important contributors to postprandial hyperglycemia in patients with type 1 diabetes.[13,14] This is partly attributed to the fact that exogenously administered insulin does not restore normal postprandial insulin concentrations in the portal vein, resulting in an abnormally elevated portal glucagon/insulin ratio. The results of the present studies demonstrate that pramlintide, an analog of the human β-cell hormone amylin, prevents an abnormal rise in postprandial glucagonemia in patients with type 1 diabetes, thus confirming previous findings of a glucagonostatic effect in rodents.[12] In conjunction with the previously demonstrated effect of pramlintide to regulate the rate of gastric emptying in patients with type 1 diabetes,[17] this meal-related glucagonostatic effect likely contributes to the effect of pramlintide to limit postprandial glucose excursions when used as an adjunct to prandial insulin therapy.[18]

When assessing postprandial glucagonemia, it is important to consider that both insulin and glucose have inhibitory effects on glucagon secretion.[19-23] To evaluate whether the observed effect of pramlintide on postprandial glucagonemia was confounded by concomitant changes in glycemia and insulinemia, Gedulin et al controlled these variables in hyperinsulinemic-euglycemic clamp experiments in rodents.[24] They demonstrated that the effect of pramlintide to suppress nutrient-stimulated glucagon secretion was independent of prevailing glucose and insulin concentrations.[24] In the present studies, glucose and insulin concentrations were not controlled. However, due to the small variation in mealtime insulin doses, it is unlikely that circulating insulin influenced the results. Moreover, the effect of pramlintide to reduce postprandial glucagon secretion occurred in the face of reduced glycemic excursions, which, if anything, would tend to increase glucagon concentrations.

It is also noteworthy that systemic plasma glucagon concentrations were measured in the present study. It appears likely that the pramlintide-induced suppression of endogenous glucagon secretion is most pronounced in the portal circulation, where the glucagon/insulin ratio and thus, the hormonal stimulus to hepatic glucose output, is reduced. This is not achievable with current modes of SC insulin therapy.

The postprandial glucagonostatic effect of pramlintide in study 2 was evident at the lowest dose tested (30 μg 3 times daily). This dose regimen, shown to improve long-term glycemic control when used as an adjunct to insulin in patients with type 1 diabetes,[8] produced a peak peripheral venous plasma pramlintide concentration of approximately 25 pmol/L, which is well within the range of postprandial peripheral venous plasma amylin concentrations in healthy subjects.[4,25] This is in agreement with findings in rodents where the glucagonostatic effect of amylin was observed at systemic plasma concentrations within the normal range as well.[24] It can be concluded, therefore, that the effect of pramlintide on postprandial glucagon secretion is of physiologic relevance and is not simply a pharmacologic effect. This, in turn, raises the possibly that the abnormal postprandial rise in glucagonemia frequently seen in patients with type 1 diabetes treated with insulin alone[26] may, at least in part, be attributable to their absolute amylin deficiency. Although no human data are yet available, preclinical findings that administration of a selective amylin antagonist to rodents increases circulating glucagon concentrations[27] support a physiologic role of endogenous amylin in the regulation of glucagon secretion.

Another important finding was that the postprandial glucagonostatic effect of pramlintide was evident upon both single infusion and 14-day SC injections, indicating that the effect is sustained and not just a transient phenomenon. This agrees with the previous finding that pramlintide administered subcutane-

ously for 4 weeks to patients with type 1 diabetes reduced plasma glucagon concentrations in response to a carbohydrate-rich breakfast.[18]

Nyholm et al reported that during insulin-induced hypoglycemia, pramlintide did not compromise secretion of glucagon or other counter-regulatory hormones indicating the presence of a "hypoglycemic override" mechanism.[28] This agrees with findings in rodents where the glucagonostatic effect of amylin was shown to be absent in the presence of insulin-induced hypoglycemia.[12] A similar override mechanism has previously been demonstrated for pramlintide's effect on gastric emptying.[29] Collectively, these results are consistant with the idea that the glucagonostatic effect of pramlintide does not compromise the physiologic defense against hypoglycemia. This is in accordance with findings from a recent meta-analysis of long-term clinical studies in more than 1,000 patients with type 1 diabetes, showing that pramlintide as an adjunct to insulin therapy facilitates achievement of glycemic targets without increasing the overall event rate of severe hypoglycemia.[30]

The exact mechanism by which amylin and pramlintide suppress nutrient stimulated glucagon secretion has not yet been fully elucidated. Binding studies with radioiodinated amylin in rodents and primates have failed to demonstrate amylin binding in pancreatic alpha cells, making a direct effect unlikely. Instead, high-affinity amylin binding sites have been identified in distinct regions of the brain, including the *nucleus accumbens*, *dorsal raphe*, and *area postrema*.[31] The *area postrema*, which is a part of the dorsal vagal complex and one of the few brain regions lacking a blood-brain barrier (and therefore exposed to circulating glucose and glucoregulatory hormones), appears to be important in bringing about the effect of amylin. In rodents with a targeted lesion of the *area postrema*[32] or with a bilateral subdiaphragmatic vagotomy[33] the effect of amylin on gastric emptying is absent. This is consistent with amylin acting as a neuroendocrine hormone via which β cells partake in the central regulation of postprandial nutrient influx. Although it is presently unknown whether the glucagonostatic effects of amylin and pramlintide are also centrally mediated, recent studies in the perfused rat pancreas have clearly demonstrated that the effect is extrapancreatic.[12] Since the Sustacal test meal had a relatively high protein content (24% of the calories), the observed glucagonostatic effect of pramlintide might, at least in part, be secondary to its effect on gastric emptying. Although the present study cannot rule out this possibility altogether, preclinical findings that amylin/pramlintide lowers glucagon concentrations under euglycemic clamp conditions and in response to an intravenous arginine stimulus[24] clearly indicate that pramlintide's glucagonostatic effect is not simply a secondary consequence of its effect on gastric emptying.

In summary, the results of the present studies demonstrate that mealtime amylin replacement with pramlintide, as an adjunct to insulin, prevents the abnormal postprandial plasma glucagonemia seen in patients with type 1 diabetes treated with insulin alone. In conjunction with the previously demonstrated effect on gastric emptying in patients with type 1 diabetes,[9] this meal-related glucagonostatic effect likely contributes to the effect of pramlintide to limit postprandial glucose excursions and improve long-term glycemic control when used as an adjunct to insulin therapy.

ACKNOWLEDGMENT

We thank Tracy Quinten and Bonnie Tweed for their excellent administrative assistance and Eric Blase for data management.

REFERENCES

1. Moore CX, Cooper GJS: Co-secretion of amylin and insulin from cultured islet beta-cells: Modulation by nutrient secretagogues, islet hormones and hypoglycemic agents. Biochem Biophys Res Commun 179:1-9, 1991

2. Hartter E, Svoboda T, Ludvik B, et al: Basal and stimulated plasma levels of pancreatic amylin indicate its co-secretion with insulin in humans. Diabetologia 34:52-54, 1991

3. Young A, Moore C, Herich J, et al: Neuroendocrine actions of amylin, in Poyner D, Marshall I, Brain S (eds): Calcitonin Gene-Related Peptide (CGRP). Austin, TX, Landes, 1999, pp 91-102

4. Weyer C, Maggs DG, Young AA, et al: Amylin replacement with pramlintide as an adjunct to insulin therapy in type 1 and type 2 diabetes mellitus: A physiological approach toward improved metabolic control. Curr Pharm Des 7:1353-1373, 2001

5. Koda JE, Fineman M, Rink TJ, et al: Amylin concentrations and glucose control. Lancet 339:1179-1180, 1992

6. Gottlieb A, Velte M, Fineman M, et al: Pramlintide as an adjunct to insulin therapy improved glycemic and weight control in people with type 1 diabetes during treatment for 52 weeks. Diabetes 49:A109, 2000 (abstr)

7. Fineman M, Gottlieb A, Bahner A, et al: Pramlintide therapy in addition to insulin in type 1 diabetes: Effect on metabolic control after 6 months. Diabetologia 42:A232, 1999 (abstr 0872)

8. Whitehouse F, Kruger DF, Fineman M, et al: A randomized study and open-label extension evaluating the long-term efficacy of pramlintide as an adjunct to insulin therapy in type 1 diabetes. Diabetes Care (in press)

9. Kong MF, King P, Macdonald IA, et al: Infusion of pramlintide, a human amylin analogue, delays gastric emptying in men with IDDM. Diabetologia 40:82-88, 1997

10. Young AA, Vine W, Gedulin BR, et al: Preclinical pharmacology of pramlintide in the rat: Comparisons with human and rat amylin. Drug Dev Res 37:231-248, 1996

11. Rayner CK, Samsom M, Jones KL, et al: Relationships of upper gastrointestinal motor and sensory function with glycemic control. Diabetes Care 24:371-381, 2001

12. Silvestre RA, Rodriguez-Gallardo J, Jodka C, et al: Selective amylin inhibition of the glucagon response to arginine is extrinsic to the pancreas. Am J Physiol 280:E443-E449, 2001

13. Unger RH, Orci L: The role of glucagon in the endogenous hyperglycemia of diabetes mellitus. Annu Rev Med 28:119-130, 1977

14. Unger RH, Aguilar-Parada E, Muller WA, et al: Studies of pancreatic alpha cell function in normal and diabetic subjects. J Clin Invest 49:837-848, 1970

15. Abourizk NN, Dunn JC: Types of diabetes according to National Diabetes Data Group classification—Limited applicability and need to revisit. Diabetes Care 13:1120-1123, 1990

16. Slein MW, Cori GT, Cori CF: A comparative study of hexokinase from yeast and animal tissues. J Biol Chem 186:763-780, 1950

17. Kong MF, Stubbs TA, King P, et al: Effect of single doses of pramlintide on gastric emptying of two meals in men with IDDM. Diabetologia 41:577-583, 1998

18. Nyholm B, Orskov L, Hove KY, et al: The amylin analog pramlintide improves glycemic control and reduces postprandial glu-

cagon concentrations in patients with type 1 diabetes mellitus. Metabolism 48:935-941, 1999

19. Pipeleers DG, Schuit FC, Van Schravendijk CF, et al: Interplay of nutrients and hormones in the regulation of glucagon release. Endocrinology 117:817-823, 1985

20. Samols E, Marri G, Marks V: Interrelationship of glucagon, insulin, and glucose. The insulinogenic effect of glucagon. Diabetes 15:855-866, 1966

21. Starke A, Grundy S, McGarry JD, et al; Correction of hyperglycemia with phlorizin restores the glucagon response to glucose in insulin-deficient dogs: Implications for human diabetes. Proc Natl Acad Sci USA 82:1544-1546, 1985

22. Raskin P, Fujita Y, Unger RH: Effect of insulin-glucose infusions on plasma glucagon levels in fasting diabetics and nondiabetics. J Clin Invest 56:1132-1138, 1975

23. Muller WA, Faloona GR, Unger RH: The effect of experimental insulin deficiency on glucagon secretion. J Clin Invest 50:1992-1999, 1971

24. Gedulin BR, Rink TJ, Young AA: Dose-response for glucagonostatic effect of amylin in rats. Metabolism 46:67-70, 1997

25. Young A: Amylin's physiology and its role in diabetes. Curr Opin Endocrinol Diabetes 4:282-290, 1997

26. Scherbaum WA: The role of amylin in the physiology of glycemic control. Exp Clin Endocrinol Diabetes 106:97-102, 1998

27. Gedulin B, Jodka C, Young A: Studies using neutralizing antibody and the antagonist AC187 reveal that endogenous amylin inhibits glucagon secretion. Diabetologia 40:A300, 1997 (abstr 1179)

28. Nyholm B, Moller N, Gravholt CH, et al: Acute effects of the human amylin analog AC137 on basal and insulin-stimulated euglycemic and hypoglycemic fuel metabolism in patients with insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 81:1083-1089, 1996

29. Gedulin BR, Young AA: Hypoglycemia overrides amylin-mediated regulation of gastric emptying in rats. Diabetes 47:93-97, 1998

30. Maggs D, Fineman M, Burrell T, et al: In type 1 diabetes, addition of pramlintide to insulin therapy resulted in long-term glycemic imrprovement without the increase in severe hypoglycemia noted in patients treated with insulin alone. Diabetes 50:A442, 2001 (abstr)

31. Sexton PM, Paxinos G, Kenney MA, et al: In vitro autoradiographic localization of amylin binding sites in rat brain. Neuroscience 62:553-567, 1994

32. Edwards GL, Gedulin B, Jodka C, et al; Area postrema (AP)-lesions block the regulation of gastric emptying by amylin. Neurogastroenterol Motil 10:26, 1998 (abstr)

33. Jodka C, Green D, Young A, et al: Amylin modulation of gastric emptying in rats depends upon an intact vagus nerve. Diabetes 45: 235A, 1996 (abstr)