The Role of Amylin and Glucagon in the Dampening of Glycemic Excursions in Children With Type 1 Diabetes

Rubina A. Heptulla, Luisa M. Rodriguez, Lisa Bomgaars, and Morey W. Haymond

Postprandial hyperglycemia and preprandial hypoglycemia contribute to poor glycemic control in type 1 diabetes. We hypothesized that postprandial glycemic excursions could be normalized in type 1 diabetes by suppressing glucagon with pramlintide acetate in the immediate postprandial period and supplementing glucagon in the late postprandial period. A total of 11 control subjects were compared with 8 type 1 diabetic subjects on insulin pump therapy, using the usual insulin bolus–to–carbohydrate ratio during a standard liquid meal. Type 1 diabetic subjects were then randomized to two open-labeled studies. On one occasion, type 1 diabetic subjects received a 60% increase in the insulin bolus–to–carbohydrate ratio with minidose glucagon rescue injections, and on the other occasion type 1 diabetic subjects received 30–45 μg pramlintide with their usual insulin bolus–to–carbohydrate ratio. Glucose, glucagon, amylin (pramlintide), and insulin concentrations were measured for 420 min. The plasma glucose area under the curve (AUC) for 0–420 min was lower in control versus type 1 diabetic subjects (316 ± 5 vs. 929 ± 18 mg · h⁻¹ · dl⁻¹, P < 0.0001). Pramlintide, but not an increase in insulin, reduced immediate postprandial hyperglycemia (AUC₀alog 470 ± 43 vs. 434 ± 48 mg · h⁻¹ · dl⁻¹, P < 0.01). Pramlintide administration suppressed glucagon (P < 0.02), and glucagon injections prevented late hypoglycemia with increased insulin. In summary, in type 1 diabetes, glucagon modulation with pramlintide as an adjunct to insulin therapy may prove beneficial in controlling postmeal glycemic swings. Diabetes 54:1100–1107, 2005

The landmark Diabetes Control and Complications Trial (DCCT) demonstrated that improving glycemic control for patients with type 1 diabetes prevented or delayed the onset of long-term microvascular complications (1). Since the DCCT, intensive therapy has been directed at achieving glucose and HbA₁c values as close to normal as safely possible. However, hyper- and hypoglycemia continue to be problematic in the management of type 1 diabetes, especially in children (2–6).

Type 1 diabetes is presumed to be caused by the autoimmune destruction of the insulin-secreting β-cells of the pancreas. Besides β-cells, it is thought that other hormones, such as glucagon and amylin, may play a role in normalizing glucose excursions. Failure of glucagon suppression results in immediate postprandial hyperglycemia, and a loss of glucagon response to hypoglycemia results in late postprandial hypoglycemia (7,8). Despite the increasing use of subcutaneous continuous insulin administration (2,9) and newer insulin analogs, insulin replacement remains imperfect, and glucose excursions are not adequately normalized in diabetes (10). This suggests that other factors in addition to diet and insulin management may need to be addressed if postprandial glucose excursions are to be normalized in type 1 diabetes (11).

The recent discovery of the hormone amylin has enhanced our understanding of postprandial glucose homeostasis (12). Patients with type 1 diabetes have deficiencies of both insulin and amylin (13). Amylin, a 37-amino acid polypeptide hormone, is cosecreted from the pancreatic β-cells in conjunction with insulin in response to nutrient stimuli (14). Amylin, in the immediate postprandial period, may mediate part of its effect by suppressing glucagon secretion, resulting in the suppression of hepatic glucose production (15) and the slowing of gastric emptying (15,16). It remains unclear whether these two actions of amylin, namely glucagon suppression and gastric emptying, are independent of each other, or if one could be the result of the other. Pramlintide acetate is a synthetic analog of the naturally occurring human hormone amylin. It is soluble, nonaggregating, and is generated by replacing three amino acid residues of the amylin molecule. It effectively reproduces amylin agonist activity in an equipotent fashion (17). Pramlintide acetate has been reported to improve glycemic control in adults with both type 1 and type 2 diabetes (17,18). Specifically, postprandial glucose excursions are improved with adjunctive pramlintide use compared with insulin alone (17,19). To our knowledge, there are no studies examining amylin excursions in children with and without diabetes and/or amylin replacement.

Late postprandial and nocturnal hypoglycemia continues to be problematic in children with type 1 diabetes (2,4). Hypoglycemia was increased threefold in the DCCT study and is the major limiting factor in achieving “tight” metabolic control in type 1 diabetes, regardless of age or treatment modality (1). A recent study by Bulsara et al. (6)
reported that severe hypoglycemia continues to be a major problem for children and adolescents with type 1 diabetes. Furthermore, there was no significant difference in hypoglycemia rates between those treated with analogs and those treated with regular insulin. A previous study from our laboratory suggests that subcutaneous administration of minidoses of glucagon rescue injections prevents hypoglycemia and results in a sustained rise in glucose for at least 1 h in children with nausea and vomiting (20). Thus, a two-pronged approach using pramlintide to prevent immediate postprandial hyperglycemia and increased insulin with minidose glucagon rescue could provide new strategies to normalize glucose excursions in children with type 1 diabetes.

The current study was designed to examine the role of amylin and glucagon in postprandial glucose homeostasis in children with and without type 1 diabetes. To address immediate postprandial hyperglycemia and late postprandial hypoglycemia, the following hypotheses were tested: 1) pramlintide acetate, as an adjunct to insulin before meals, will prevent immediate postprandial hyperglycemia more effectively than increasing insulin bolus for meals, and 2) minidose glucagon rescue injections in the late postprandial period will prevent late postprandial hypoglycemia, thus restoring euglycemia in children with type 1 diabetes.

RESEARCH DESIGN AND METHODS
The institutional review board of the Baylor College of Medicine approved this investigator-initiated study. Amylin Pharmaceuticals played no role in the design, implementation, or interpretation of data.

From our population of 1,200 patients with type 1 diabetes, we approached patients aged 12–18 years who were on subcutaneous insulin pump therapy. At the screening visit, a detailed history and physical exam were performed. Subjects had normal BMI (<90th percentile for age), their hemoglobin was ≥12 g/dl, and they were in moderate glycemic control, with HbA1c <8%. They had no other chronic conditions besides diabetes and/or hypothyroidism and were not on medications that affect glucose concentrations. Pregnant and lactating women were excluded from the study. Control subjects were healthy relatives of children with diabetes or individuals that have participated as control subjects at the Children’s Nutrition Research Center at Baylor College of Medicine. Control subjects were Tanner stage—age, and sex-matched to those with type 1 diabetes.

A total of 18 subjects with type 1 diabetes and 12 control subjects were screened. Of 21 subjects with type 1 diabetes and 11 control subjects qualified for the study. All control subjects completed the study. Eight control subjects at the Children’s Nutrition Research Center at Baylor College of Medicine were provided by Amylin Pharmaceuticals, the pramlintide acetate premeal dose was the same as the that administered in study A. Based on guidelines provided by Amylin Pharmaceuticals, the pramlintide acetate premeal dose was 45 µg for the subjects with insulin requirements of >1 unit/kg/day and 30 µg in subjects with insulin requirements of <1 unit/kg/day. Blood samples were drawn before study start. The subjects then received an insulin bolus just before the test meal, which was based on a standard meal and their usual insulin bolus-to-carbohydrate ratio. The average insulin bolus was 5 ± 0.7 units. At time 0, subjects drank 12 oz of Boost high-protein drink (360 calories, 50 g carbohydrates, 22 g fat) over a period of 10 min. Blood samples for hormone analysis (insulin, amylin, and glucagon) were drawn at 10- to 30-min intervals for 420 min. Blood glucose was measured at the bedside using a YSI glucose analyzer at 10-min intervals from 40 min to 420 min. During the study, intravenous glucose (5–7 g) was given if glucose was <3 mmol/L. Control subjects underwent study A in exactly the same fashion as the type 1 diabetic subjects, except they did not receive insulin.

Study B (insulin + pramlintide). Only subjects with type 1 diabetes participated in study B. The study was conducted in a fashion identical to study A, except for the addition of a separate subcutaneous injection of company-provided pramlintide acetate (Symelin, Amylin Pharmaceuticals, San Diego, CA) just before test meal (0 min). The insulin dose for the meal bolus was the same as the that administered in study A. Based on guidelines provided by Amylin Pharmaceuticals, the pramlintide acetate premeal dose was 45 µg for the subjects with insulin requirements of >1 unit/kg/day and 30 µg in subjects with insulin requirements of <1 unit/kg/day. Blood draws were similar to study A in every respect, except instead of amylin measurements, pramlintide plasma concentrations were obtained. Blood samples (5 ml) were obtained before pramlintide dosing and at 20, 40, 60, 120, 180, 240, and 330 min after drug administration. After collection, samples were immediately centrifuged at 5,000g for 5 min and stored at −20°C or lower until analysis. During the study, intravenous glucose (5–7 g) was given if blood glucose was <3 mmol/L. If blood glucose did not improve in 20 min, the dose was repeated for a maximum of three doses. Insulin adjustments were not made between 0 and 420 min.

Study C. Study C was identical to study A, with the exception of a 60% increase in the insulin bolus and the use of minidose glucagon for hypoglycemia correction. The bolus was administered just before the meal at 0 min. If a subject’s blood glucose fell below 5.3 mmol/L, a minidose of glucagon (commercially available) was administered subcutaneously as a bolus injection based on their age. Using a standard U-100 insulin syringe, a dose of 1 unit/year of age (each unit equal to 10 µg) up to a maximum dose of 15 units (150 µg) was administered. If the blood glucose level did not increase within 30 min, the initial dose was doubled and given again (20).

Measurements. Blood glucose concentrations were measured using a glucose analyzer (2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH). A DCA 2000 HbA1c system (Bayer, Elkhart, Indiana) was used for measuring the percentage concentration of HbA1c in blood. HbA1c concentrations in the range of 2.5% to ~14.0% are reported. An ultrasensitive radioimmunoassay (RIA) measured plasma insulin, which determines ultralatrate insulin with a detection limit of 0.5 µU/mL. Glucagon was measured by RIA, using an antibody specific to pancreatic glucagon. Human plasma amylin was measured by an enzyme-linked immunosorbent assay monoclonal antibody—

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<th>TABLE 1 Clinical characteristics</th>
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<td>Age (years)</td>
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<td>Sex (M/F)</td>
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<td>Duration of diabetes (years)</td>
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<td>Height (m)</td>
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<td>Diastolic blood pressure (mmHg)</td>
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<td>Pulse (bpm)</td>
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Data are means ± SE.
based immunoassay. The capture antibody recognizes human amylin, amylin acid (deamidated amylin), and a 1–20 fragment of amylin. It has sensitivity to 1 pmol/l, a standard range of 1–100 pmol/l, 99% specificity for amylin, and ≤1% cross-reactivity with glucagon-like peptide 1, adrenomedullin, glucagon, and insulin. All RIA kits used were purchased from Linco Research (St. Charles, MO). Blood samples were analyzed for pramlintide acetate by Amylin Pharmaceuticals, using a previously described immunoradiometric assay (21). The lower limit of quantification for pramlintide acetate was 2.5 pmol/l.

Statistics. Repeated-measures ANOVA was used to analyze glucose and hormonal excursions for the three studies. Significance was considered at a 0.05 level, and post hoc analysis using paired two-tailed student t tests (paired analysis for type 1 diabetes and independent groups for control versus type 1 diabetes) was applied. GraphPad Prism 4.0 was used for data analysis.

Pharmacokinetic data analysis. Plasma concentration–time data were modeled in ADAPT II (22). Both one- and two-compartment models were fitted to the data. Akaike’s Information Criterion (23) was used to determine the best fit. Pharmacokinetic parameters, including clearance, half-life, and volume of distribution at steady state, were derived from estimates of the model parameters. The area under the curve (AUC) for concentration versus time was calculated using the linear trapezoidal rule, and the residual area from the last quantifiable concentration to infinity was calculated using the approximation \( AUC_{\infty} = \frac{C_{\text{last}}}{k_{\text{el}}} \), where \( k_{\text{el}} \) is the apparent terminal elimination rate constant determined by log-linear regression of the terminal log-linear segment of the plasma concentration–time curve. Pharmacokinetic results are descriptive.

RESULTS

Control versus type 1 diabetic subjects (study A). Figure 2 depicts the plasma concentrations of glucose, insulin, amylin, and glucagon excursions in children with and without diabetes before and after mixed meal ingestion. Type 1 diabetic subjects had marked elevations in glucose (\( P < 0.0001 \)) after the mixed meal compared with baseline, as opposed to subjects with type 1 diabetes, who had a modest twofold increase (Fig. 2B). Although baseline plasma amylin concentrations were detected in five of the eight subjects, they did not increase in response to a meal, as was observed in the control subjects (Fig. 2C). Glucagon concentrations did not differ in children with and without type 1 diabetes on ingestion of a mixed meal (\( P < 0.4 \)) (Fig. 2D).

Type 1 diabetes

Study A versus study B. Pramlintide markedly reduced immediate postprandial plasma glucose (Fig. 4) concentrations in type 1 diabetic subjects after a mixed meal (\( P < 0.0001 \)). A nadir of 4.75 mmol/l occurred at 70 min post–meal ingestion. Blood glucose concentrations <3.6 mmol/l occurred in five of the eight subjects who received pramlintide, four of whom had received the higher dose of pramlintide (45 \( \mu \)g), and three of these received intravenous glucose (5–7 g) in the immediate postprandial period to prevent further lowering of plasma glucose. One of the two subjects who received 30 \( \mu \)g pramlintide experienced low plasma glucose concentrations and needed to be treated with intravenous glucose. After the initial nadir in immediate postprandial glucose concentrations, plasma glucose continued to increase, reaching a maximum of 9 mmol/l at 220 min.

Pramlintide pharmacokinetic analysis. All patients who completed study B (\( n = 8 \)) had samples obtained for pramlintide acetate pharmacokinetic analysis, as outlined in Table 2. All patients had plasma levels above the lower limits of detection at the 120-min time point, six had detectable levels at 180 min, and only one patient had detectable levels at 300 min. The \( AUC_{\infty} \) value (means ±
In the six patients receiving the 45-μg dose was 4,159 \pm 406 \text{ vs. } 8,459 \text{ and } 3,620 \text{ pmol } \text{ min}^{-1} \cdot \text{L}^{-1} \text{ in the two patients evaluated at the } 30-\mu g \text{ dose. In three patients, a two-compartment model provided a better fit. In patients' best fit using the two-compartment model, the } t_{1/2} \text{ was similar to the terminal half-life in patients fit with a one-compartment model. In patients with detectable late time points, a prolonged } t_{1/2} \text{ is suggested. Figure 5 demonstrates pharmacodynamic effects of pramlintide, with glucagon suppression and glucose suppression occurring during peak pramlintide action. As shown in Fig. 6, compared with the baseline study, pramlintide suppressed glucagon significantly (} P < 0.02 \text{ after the ingestion of a mixed meal in type 1 diabetic subjects.}

**Study A versus study C.** Increasing the insulin bolus by 60% (Fig. 4B) did not significantly decrease the postprandial period (} P = \text{NS}). The AUC for glucose was no different from baseline when the insulin bolus was increased in type 1 diabetic subjects (Fig. 3C and D). An average of two glucagon rescue injections were required to prevent hypoglycemia. Interestingly, all of the male subjects required rescue glucagon injections with the increased dose of insulin; however, the two female subjects did not require glucagon rescue. As anticipated, glucagon (} P < 0.03 \text{) concentrations were higher in study C (Fig. 6) compared with the basal study. However, insulin concentrations were not different from the baseline study (} P < 0.8).

**Study B versus study C.** Pramlintide (} P < 0.0004 \text{) was more effective than increasing insulin bolus before meals in curtailing the immediate postprandial glucose concentration.

**DISCUSSION**

These investigations demonstrate that postprandial hyperglycemia contributes significantly to poor glycemic control in children with type 1 diabetes compared with control subjects. Despite the use of insulin pump therapy, immediate and prolonged (lasting 3 h) hyperglycemia persists. Moreover, accurately counting carbohydrates and giving insulin before the meal does not effectively control blood glucose concentrations after a meal. A very mild decrease in glucose concentrations occurred when the insulin bolus was increased, as compared with the response noted when pramlintide was administered with insulin. Also of note, the higher dose of insulin would likely have resulted in significant hypoglycemia without the use of minidose rescue glucagon injections postmeal. This highlights the dilemma patients face when trying to normalize postprandial hyperglycemia with insulin alone: late hypoglycemia is an unwanted but invariable consequence of increasing the preprandial insulin dose. Pramlintide coadministration with insulin resulted in an immediate lowering of blood glucose in all subjects from baseline, with a nadir occurring at 45–60 min. Our data (Fig. 6) are consistent with previous reports and are likely mediated in part by glucagon suppression (15). However, pramlintide is also known to slow gastric emptying (16). Because we did not assess gastric emptying in this study, we cannot speculate what the relative contribution of these two mechanisms may be in inducing a glucose-lowering effect. In future studies, it will be important to study the effect of pramlintide on gastric emptying so as to distinguish the relative contribution of gastric emptying versus the glucagon-suppressive effects of pramlintide.

Adjunctive pramlintide therapy has resulted in improvement in postprandial glucose excursion and is reported in a several studies (24,25). Our study is the first to examine
the pharmacodynamics and pharmacokinetics of pramlintide in adolescents with type 1 diabetes. Postprandial hypoglycemia was noted in four of six patients receiving the 45-µg dose and one of two patients receiving the 30-µg dose. We did not see a correlation between pramlintide AUC and the development of hypoglycemia (4,693 ± 794 pmol · min⁻¹ · l⁻¹ in hypoglycemic patients and 4,572 ± 300 pmol · min⁻¹ · l⁻¹ in normoglycemic patients).

The higher dose (45 µg) of pramlintide was chosen in some of the patients because adolescents with type 1 diabetes are insulin resistant and require a higher insulin dose than adults (26). In keeping with the insulin needs, pramlintide dosages were increased as recommended by Amylin Pharmaceuticals. Currently, there is no weight-based pramlintide dosing information available. Hence, we believe we may have overestimated the dose of pramlintide, resulting in immediate postprandial hypoglycemia in some of the subjects. Whitehouse et al. (27) have suggested that rapid-acting insulin analogs such as lispro may additionally cause this nadir of glucose in the immediate postprandial period and that a lowering of insulin dosage by 30–50% may be necessary to prevent immediate postprandial hypoglycemia when simultaneously using pramlintide. A previously unreported effect of pramlintide injection noted in our study was an escape phenomenon. Blood glucose levels in all subjects continued to rise after the immediate lowering of postprandial glucose. This may be attributable to the waning effect of pramlintide resulting in increased gastric emptying and/or glucagon secretion.

Pramlintide was generally very well tolerated. Known side effects of pramlintide therapy, other than hypoglycemia, are nausea and vomiting (27). In this study, one patient had vomiting after pramlintide administration and opted out of the study. One other patient complained of nausea, but he continued with the study without emesis. The others reported no symptoms. As expected, preprandial hypoglycemia occurred during the baseline study in three type 1 diabetic subjects toward the end of the baseline study (study A). There were no side effects noted with minidose glucagon injection. In one patient, four glucagon injections were required to restore euglycemia. However, most subjects required only one to two injections. In all instances, a glycemic response to glucagon was observed after 120–150 µg glucagon s.c., and maybe an even smaller dose than the one suggested by Haymond and Schreiner (20) would have sufficed.

The limitations of our study are that most children are active during the day, and the hyperglycemia noted in study A was exaggerated because they were in bed for the entire study period. Conversely, during the study, accurate assessment of carbohydrates and the insulin dose did not correct marked postprandial hyperglycemia. Subjects with type 1 diabetes are not usually very rigorous in counting carbohydrates and may underdose or even forget to administer insulin boluses preprandially (28), thus worsening hyperglycemia. Minidose glucagon was very effective in preventing late postprandial hypoglycemia; however, it is difficult to incorporate into daily life. Separate glucagon injections that have to be titrated to accurate glucose concentrations makes glucagon a less attractive therapeutic modality. However, with the advent of closed-loop insulin delivery devices with concurrent continuous glucose monitoring, hypoglycemia could be prevented by incorporating low-dose subcutaneous glucagon infusion (29,30).

Our results suggest that there is marked postprandial hyperglycemia even in well-controlled patients with type 1 diabetes and that insulin pump therapy alone does not correct postprandial hyperglycemia. Even increasing the insulin dose before a meal does not correct immediate postprandial hyperglycemia. Pramlintide is effective in decreasing immediate postprandial hyperglycemia in
adolescents with type 1 diabetes. Minidose glucagon is effective in preventing late postprandial hypoglycemia. Therefore, we conclude that multiple hormones contribute to normal glucose homeostasis in type 1 diabetes. Further refinement in our understanding as to how to replace or correct the action of these hormones will result in decreased glycemic swings and will allow lowering of HbA1c into the true normal range without the increased risk of hypoglycemia.

FIG. 4. A: Plasma glucose concentrations in children with type 1 diabetes after a mixed meal and usual insulin bolus, with (study B, □) and without (study A, □) pramlintide administration. B: Plasma glucose concentrations in type 1 diabetes with administration of usual insulin bolus for fixed meal (study A, □) and 60% increase in insulin bolus and rescue with mini-glucagon injections to prevent hypoglycemia (study C, □). Data are means ± SE. mcg, microgram; S/C, subcutaneous.

FIG. 5. Plasma pramlintide pharmacokinetics and pharmacodynamics on glucose and glucagon concentrations in subjects with type 1 diabetes. Data are means ± SE. mcg, microgram.

Table 2
Pharmacokinetic parameters for pramlintide after injection of 30 or 45 µg i.p.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All dose levels</th>
<th>30 µg (n = 2)</th>
<th>45 µg (n = 6)</th>
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<tbody>
<tr>
<td>Clearance (1 · min⁻¹ · kg⁻¹)</td>
<td>0.0553 ± 0.03</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Volume of distribution (l/kg)</td>
<td>2.2 ± 0.3</td>
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<tr>
<td>t₁/₂α (min)</td>
<td>34 ± 6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>t₁/₂β (min)†</td>
<td>33 ± 10</td>
<td>—</td>
<td>—</td>
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<tr>
<td>AUCₚ (pmol · min⁻¹)</td>
<td>6,040 ± 2,219</td>
<td>4,159 ± 406</td>
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Data are the means ± SE. *Patients with two-compartment best fit (n = 3); †patients with one-compartment best fit (n = 5).
ACKNOWLEDGMENTS

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FIG. 6. Insulin and glucagon in study A versus study B (top panels) and study A versus study C (bottom panels). Panels show changes in glucagon and insulin excursions in children with type 1 diabetes comparing pramlintide administration (○) and usual insulin dose of insulin without pramlintide (□) or usual insulin dose (□) with a 60% increase in insulin dose (□).


