Pramlintide, an amylin analog, selectively delays gastric emptying: potential role of vagal inhibition

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The aim of the present study was to compare the effects of a 5-day treatment with placebo or 30 or 60 µg (tid) of pramlintide on gastrointestinal and colonic transit in healthy volunteers and to investigate the role of pramlintide on gastric emptying by measuring the plasma pancreatic polypeptide response to a standard meal. The first-hour postprandial response in plasma pancreatic polypeptide was previously used as a surrogate of gastric emptying (3, 20). In two previous studies in healthy subjects from different laboratories, the incre-

amylin is a 37-amino acid peptide cosecreted with insulin by the pancreatic β-cells in response to nutrient intake (29). Immunoassays of amylin in healthy subjects showed fasting amylin concentrations between 4 and 8 pmol/l. These levels increased two- to threefold after the ingestion of a mixed meal (14). In patients with type I diabetes mellitus, amylin concentrations either are at the lower end of detection of the assay or are undetectable; circulating levels of amylin do not increase after nutrient stimulation (14). In contrast, the levels of amylin in type II diabetes parallel those of insulin (9), suggesting that the two are cosecreted from the pancreatic islet β-cells.

Animal studies indicate that amylin slows the rate of nutrient delivery to the small intestine by inhibition of gastric emptying; this inhibition of gastric emptying is avoided during insulin-induced hypoglycemia, which is associated with vagal stimulation (12, 26–28). Postprandial glucagon secretion is inhibited during amylin-induced retardation of gastric emptying (10). This may further enhance glycemic control postprandially.

Pramlintide is a stable, bioactive peptide analog of amylin that differs in three amino acids (19). Doses of pramlintide that are associated with amylin levels in the physiological range attenuate postprandial glucose concentrations in patients with type I diabetes mellitus (15). There is increasing evidence that pramlintide improves glycemic control in both type I and type II diabetes mellitus (24, 25). Thus Thompson et al. (24) showed that 4-wk administration of pramlintide at doses of 30 or 60 µg, three or four times per day, improved blood glucose profiles and fructosamine concentrations in 215 type I diabetic patients. Similar results were observed in type II diabetic patients (25).

The influence of pramlintide on gastric emptying has been documented in animal studies (28). Supraphysiologic doses of pramlintide caused a marked delay in the rate of gastric emptying in humans with type I diabetes mellitus (16). More recently, single doses of 30, 60, and 90 µg of pramlintide delayed gastric emptying in humans with type I diabetes mellitus (17). It is unclear whether tachyphylaxis after repeated administration alters the effects of the amylin analog on gastric emptying, because the effect of multiple doses of pramlintide on gastric emptying in non-amylin-deficient humans has not been investigated. The mechanism of the inhibitory effect on gastric emptying in humans is unknown. Studies in rats suggested that the effects of pramlintide may be centrally mediated (12). It is also unclear whether pramlintide affects small bowel and colonic transit in humans.

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ment in pancreatic polypeptide was at least 100 pg/ml during the first postprandial hour (11, 20).

MATERIALS AND METHODS

After approval by the Mayo Clinic Institutional Review Board, 19 healthy volunteers (4 males, 14 females; mean age 35 yr (range 25–41 yr); mean body wt 79.4 kg (range 56.1–101.6 kg)) were recruited after public advertisement at Mayo Clinic. One participant withdrew from the study and was replaced with a subject who received pramlintide (60 µg tid). All subjects completed a validated questionnaire (23) to ensure that they had no gastrointestinal symptoms. Inclusion criteria included males and females 18–65 years of age, and females of childbearing potential had to have a negative pregnancy test within 24 h of the onset of treatment and again before the scintigraphic transit test.

Experimental design. The effect of pramlintide on gastrointestinal and colonic transit, glucose concentrations, and pancreatic polypeptide response to a standardized meal was evaluated in a parallel-group dose-response designed study with six subjects being randomized to each group: placebo or 30 or 60 µg (tid sc) of pramlintide. The subjects self-administered three injections of the study drug within 15 min of each meal. All participants were instructed in this technique for self-administration by the nursing staff. The injections were administered into the subcutaneous tissue of the anterior abdominal wall. The participants received medication daily for 5 days; the transit test started on day 3. As mentioned above, one participant dropped out, and during the blind phase of the study a replacement volunteer was recruited and therapy was randomly selected for this individual.

Gastrointestinal and colonic transit. An adaptation of our established scintigraphic method was used (4, 6, 7, 18). 111In adsorbed on activated charcoal particles was delivered to the colon by a methacrylate-coated delayed-release capsule (2). The capsule was ingested after an overnight fast. Within 15 min after the capsule emptied from the stomach, which was checked by its position relative to a radioisotopic marker placed on the left anterior iliac crest, a radiolabeled meal was ingested. If the capsule had not emptied from the stomach after 1 h, the medication was given, followed 15 min later by the meal, which was consumed by all subjects in <10 min. The meal consisted of two scrambled eggs labeled with 99mTc-sulfur colloid, one slice of whole wheat bread, and one glass of whole milk (330 kcal). This meal facilitated measurement of gastric emptying and small bowel transit. Subjects ingested standardized meals for lunch and dinner. The study drug was self-administered 15 min before the radiolabeled meal was eaten.

Scintigraphic images were acquired every 15 min for the first 2 h, then every 30 min for the next 2 h, and at 8, 24, and 48 h after meal ingestion. Before the transit study, an intravenous cannula was inserted in the antecubital vein of one arm. Blood samples were drawn immediately before the meal as well as 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after meal ingestion. Blood was collected in ice-chilled EDTA tubes before being centrifuged at 4°C for 10 min. Plasma was stored at −20°C until assay.

Blood glucose and pancreatic polypeptide concentrations were measured by a glucose-oxidase method (using a glucose analyzer). Immunoreactivity of pancreatic polypeptide was analyzed using a radioimmunoassay kit (13). In the same test meal and radioimmunoassay for pancreatic polypeptide, we observed a change in mean pancreatic polypeptide of 120–180 pg/ml in 10 healthy controls (11).

Data and statistical analysis. A variable region of interest was used to quantitate the counts in the stomach and in each of the four regions of the colon. The counts were corrected for radionuclide decay, tissue attenuation, and downscatter of the 111In counts in the 99mTc window (18, 23). The primary gastric emptying summaries for the comparison of gastric emptying were β and k from a power exponential model and the calculated half-time (t0.5) min from these parameters (5, 8). β Summarizes the overall shape of the curve, and k is an index of the slope of the curve. We also estimated small bowel transit time (time at which 10% of the 99mTc-labeled meal entered the colon minus the lag time (4)) and colonic geometric center at 4, 8, 24, and 48 h (6, 7, 18). The geometric center is the weighted average of counts in the different regions of the colon (ascending (AC), transverse (TC), descending (DC), rectosigmoid (RS), and stool, 1 to 5, respectively). At any time, the proportion of colonic counts in each colonic region is multiplied by its weighting factor as follows:

\[
\text{g} = \frac{\%\text{AC} \times 1 + \%\text{TC} \times 2 + \%\text{DC} \times 3 + \%\text{RS} \times 4 + \%\text{stool} \times 5}{100}
\]

The mean pancreatic polypeptide and glucose concentrations were calculated for the first hour after the meal. The change in mean pancreatic polypeptide during the first hour over fasting value was also calculated.

Statistical analysis utilized a nonparametric one-way comparison (Kruskal-Wallis test) of the dose groups. Associations between pancreatic polypeptide, glucose levels, and gastric emptying were estimated using the Spearman rank correlation. All data are expressed as means ± SE; P values < 0.05 were considered significant unless stated otherwise.

RESULTS

Nineteen subjects were enrolled in the study. One subject who was randomized to the placebo group discontinued the study prematurely; the analysis is based on the remaining 18 subjects. After the study blind was removed, six subjects received 30 µg of pramlintide (tid), seven subjects received 60 µg of pramlintide (tid), and five subjects received placebo. All subjects who received a full 5-day course of pramlintide treatment completed the transit studies.

Gastric emptying. Gastric emptying was significantly delayed in subjects receiving 30 or 60 µg of pramlintide. Figure 1 shows a typical example of the delayed gastric emptying induced by 30 µg of pramlintide; after 120 min, 72% of the meal is still retained in the stomach compared with an average of 47% for subjects receiving placebo treatment. There was a significant overall effect of pramlintide on the estimated t0.5 values (P = 0.033) and gastric emptying slopes (k parameter; P = 0.025; Fig. 2A) obtained from the power exponential model. The prolongation of the lag phase was not statistically significant (P = 0.11; Fig. 3). A dose-dependent effect of pramlintide could not be demonstrated (Fig. 2B), as shown in the detailed plots for each time point in the gastric emptying studies.

Small bowel and colonic transit. An overall effect of pramlintide on small bowel transit was not detected (P = 0.54); estimated small bowel transit times were 179 ± 37, 178 ± 21, and 185 ± 27 min during 30-µg pramlintide, 60-µg pramlintide, and placebo treatment, respectively. Figure 3 shows the effects of pram-
Pramlintide on colonic transit. Similarly, no effect of pramlintide on the geometric centers of colonic isotope measured at 4, 24, and 48 h was detected ($P > 0.5$).

Pancreatic polypeptide responses and blood glucose concentrations. No differences in basal pancreatic polypeptide concentrations were observed among subjects receiving placebo vs. pramlintide. Figure 4, A and B, shows detailed plots of plasma pancreatic polypeptide and glucose levels at each time point of measurement. In contrast, the pancreatic polypeptide concentrations in the first hour after the meal (both for the first 30 min and the first 60 min; Fig. 5A, left) were significantly different among treatment groups ($P < 0.01$); they were lower in the subjects dosed with 60 µg of pramlintide compared with placebo ($P < 0.025$, adjusted $\alpha$ for 2 pairwise comparisons of 30 and 60 µg pramlintide vs. placebo; Fig. 5B). No significant differences between the effects of the two doses of pramlintide on plasma pancreatic polypeptide levels were observed. There was a significant ($P = 0.035$) overall treatment effect on the change in plasma pancreatic polypeptide levels (mean first hour value minus fasting value). Lower values were observed with 30 and 60 µg of pramlintide.

An inverse relationship (Fig. 6) was observed between the mean pancreatic polypeptide concentrations in the first postprandial hour and gastric emptying $t_{1/2}$ [Spearman correlation coefficient ($R_s$) = 0.48; $P = 0.044$]. A similar but statistically insignificant ($P = 0.1$) rank correlation was observed for pancreatic polypeptide 1 h postprandial mean values and gastric lag times. Pancreatic polypeptide was positively correlated with the $k$ summary of gastric emptying ($R_s = 0.51, P = 0.03$).

In contrast to the effect on pancreatic polypeptide response, 30 and 60 µg of pramlintide did not influence postprandial glucose. Figure 5A, right, shows comparable mean glucose concentrations during the first hour after the meal during placebo and pramlintide treatment. Moreover, no significant correlations were ob-

Fig. 1. Abdominal scintiscans 120 min after ingestion of radiolabeled meal. Note that less isotope has emptied from the stomach with 30-µg pramlintide treatment. ASIS, radiopaque marker on anterior superior iliac spine.

Fig. 2. Summary data (means ± SE) for gastric emptying parameters. Pramlintide significantly retards gastric emptying ($k$, parameter from power exponential analysis and half-time ($t_{1/2}$) with a trend to prolongation of lag time (time for 10% solid emptying). B: mean ± SE of gastric emptying for each time point at which scans were obtained.
served between the postprandial glucose concentration and any of the gastric emptying variables.

DISCUSSION

The novel findings of the present study are, first, that pramlintide delays gastric emptying in non-amylin-deficient, healthy humans without affecting small bowel and colonic transit and, second, pramlintide attenuates the plasma pancreatic polypeptide response to a meal. The relation between the pancreatic polypeptide concentrations and the rate of gastric emptying suggests that there is efferent vagal inhibition by pramlintide because there is a significant reduction in plasma pancreatic polypeptide in the first hour when a centrally mediated response to a meal is expected.

The results of this study show that the effect of pramlintide is not attenuated after 3 days of treatment with levels that have mimicked physiological plasma levels in previous studies; the increase in $t_{1/2}$ observed was in the range of 50% retardation with both doses of pramlintide. In this study, the maximal inhibitory effect of pramlintide on gastric emptying was reached by the 30-µg dose.

In contrast to the effect of pramlintide on the gastric emptying rate, no effect of pramlintide on small bowel and colonic transit could be detected. Colonic transit was measured using an indium-labeled capsule that dissolves in the terminal ileum (17). In 3 of 18 subjects, the capsule was not emptied from the stomach before the ingestion of the technetium-labeled meal. All three subjects were dosed with 60 µg of pramlintide, which suggests that pramlintide influences postprandial as well as interdigestive motility, although this was not specifically studied and requires confirmation. This
delay in emptying of the capsule from the stomach did not affect colonic transit measurements, as shown by the similar geometric centers calculated at 4 and 8 h after meal ingestion in the three groups.

The geometric centers calculated after 4, 8, 24, and 48 h were comparable after placebo and both doses of pramlintide and were within the range of results of previous studies performed with this method in a group of 57 healthy volunteers (geometric center $= 2.7 \pm 0.2$ at 24 h and $3.9 \pm 0.1$ at 48 h). Thus pramlintide at 30 and 60 µg has no effect on colonic transit in healthy subjects.

The potential mechanisms by which pramlintide delays gastric emptying are either a direct effect on the stomach by modulating gastrointestinal hormones or an effect on the central nervous system. Because no amylin receptors have been identified in the stomach, a direct effect of amylin on the stomach seems unlikely.

A second possible mechanism by which pramlintide may exert its action on gastric emptying is potentiation of the inhibition of gastric emptying by CCK and the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). A study in diabetic BB/Wistar rats showed that amylin is a more potent inhibitor of gastric emptying than CCK and GLP-1 (27). A study in diabetic patients confirmed these results; a dose of 0.5 nmol/kg GLP-1 prolonged the lag phase for gastric emptying by $\sim 30\%$ but had no effect on $t_{1/2}$ (20). This suggests that the effect of pramlintide on gastric emptying is more likely a direct effect rather than mediated via GLP-1 and CCK.

A third possible mechanism of action of pramlintide is an effect on central control of gastric emptying. Studies in rat brain revealed the presence of amylin receptors in several regions of the brain, including the area postrema, which is one of the regions that regulate the efferent activity of the vagus nerves (1, 21). Further evidence for a vagally mediated action of pramlintide was provided by the report showing that subdiaphragmatic vagotomy in rats abolishes the response of amylin to the gastric emptying rate (12). In the present study in humans, the plasma pancreatic polypeptide response to a meal was assessed to provide further insight into the mechanism involved in the pramlintide-induced delay in gastric emptying. The results and the inverse relationship between the gastric emptying rates and the plasma pancreatic polypeptide levels suggest that vagal inhibition plays an important role in the delay in the gastric emptying rate during treatment with pramlintide. The postprandial increases in the levels of HPP with 30 and 60 µg of pramlintide were significantly lower than those reported for the placebo group in this study (median 192 pg/ml) and were also lower than the >100 pg/ml mean pancreatic polypeptide increments in the first postprandial hour in two previous studies (11, 20).

Pramlintide did not alter the normal postprandial blood glucose concentrations in healthy volunteers. This finding is in contrast to the observations in patients with type I diabetes mellitus (15, 17), in whom blood glucose was reduced by relatively high intravenous doses after 60 and 90 µg (sc) of pramlintide but not after 30 µg (sc) of pramlintide (17). Using 3-O-methylglucose as an indicator for glucose absorption, Kong et al. (17) showed a delay in glucose absorption induced by 30, 60, and 90 µg of pramlintide, indicating that either the pramlintide-induced delay in gastric

![Fig. 5. A: effect of pramlintide on plasma HPP (left; $P < 0.01$ for drug effect) and blood glucose (right; no drug effect) during first hour postprandially in healthy subjects. Note maintenance of normoglycemia and inhibition of circulating HPP. Data are means ± SE. B: effect of pramlintide on plasma HPP during first 30 min (left) and second 30 min (right) postprandially. Overall treatment effect is significant ($P \leq 0.01$). *Two pairwise comparisons show that the 60-µg dose is significantly different ($P < 0.025$) from placebo for both time periods.](http://ajpgi.physiology.org/)

![Fig. 6. Relationship of gastric emptying $t_{1/2}$ and 1-h postprandial HPP response. The significant inverse association (Spearman rank correlation, $R_s$) suggests that inhibition of first hour secretion of HPP (a surrogate of vagal inhibition) is associated with a delay in gastric emptying of solids with pramlintide treatment.](http://ajpgi.physiology.org/)
emptying or direct inhibition of glucose absorption by enterocytes affected postprandial blood glucose levels. Control of postprandial glycaemia is complex and, in healthy subjects, postprandial hepatic glucose release and insulin responses may compensate for the impaired gastric emptying to maintain euglycemia.

One potential consideration in the inhibition of postprandial release of pancreatic polypeptide is that pramlintide might directly inhibit pancreatic islet cells. Although inhibition of insulin secretion in vitro has been observed during augmentation of glucose in physiological doses, high concentrations of amylin (750 pmol/l) did not inhibit insulin response to more significant hyperglycaemia (22). Because our healthy participants did not experience any significant change in postprandial glucose, we would conclude that there was no pramlintide-induced inhibition of insulin secretion. We are unable to find any evidence that amylin directly inhibits release of pancreatic polypeptide from pancreatic islets. Hence, a direct effect of pramlintide on pancreatic islet cells secreting insulin or pancreatic polypeptide seems most unlikely.

In summary, 30 and 60 μg of pramlintide delay gastric emptying in non-amylin-deficient humans without significant effects on small bowel and colonic transit. Vagal inhibition is a potential mechanism by which pramlintide exerts its action on gastric emptying.

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