Effect of Pramlintide on Symptom, Catecholamine, and Glucagon Responses to Hypoglycemia in Healthy Subjects

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Pramlintide is an analog of the human glucoregulatory hormone amylin. Previous studies have shown no clear evidence that pramlintide modifies the response to insulin-induced hypoglycemia; however, a detailed assessment of responses at hypoglycemic thresholds has not been conducted. To further test the effect of pramlintide on symptom, catecholamine, and glucagon responses, a 3-step hypoglycemic clamp was investigated in healthy volunteers. In a randomized, double-blind, placebo-controlled, crossover study, 18 healthy subjects without diabetes received subcutaneous premeal injections of either placebo or 60 μg pramlintide 3 times daily for 5 consecutive days. On day 6, subjects received study drug with breakfast and, after a 7-hour fast, were connected to a Biostator for a 3-step, 3-hour clamp experiment (insulin infusion rate: 1.0 mU/kg/min; blood glucose targets: 70, 55, and 45 mg/dL). An intravenous (IV) infusion of pramlintide (16 μg/h) or placebo was initiated at t = 60 minutes. At the end of each 60-minute clamp step, autonomic (sweating, palpitations, hunger, etc) and neuroglycopenic (confusion, headache, odd behavior, etc) symptoms were assessed using a validated visual analog scale questionnaire. Blood samples were collected at 30-minute intervals for measurement of plasma glucose, insulin, pramlintide, catecholamine, and glucagon concentrations. Intraindividual and group mean responses showed that autonomic symptoms and plasma catecholamine and glucagon concentrations increased progressively during the clamp, with no discernible differences between pramlintide and placebo treatments. Group means for catecholamines at 60 minutes were: epinephrine 233 ± 42, 892 ± 85, 2,340 ± 302 and 202 ± 25, 774 ± 114, 2,751 ± 404 pg/mL and norepinephrine 1,138 ± 86, 1,236 ± 77, 1,721 ± 158 and 1,278 ± 108, 1,259 ± 109, 1,580 ±136 pg/mL (± SEM) for placebo- and pramlintide-treated groups at 70, 55, and 45 mg/dL glucose, respectively. Group means for glucagon were 72 ± 6.3, 98 ± 11.1, 130 ± 14.7 and 63 ± 3.6, 92 ± 9.4, 120 ± 16.0 pmol/L (± SEM) for placebo- and pramlintide-treated groups at 70, 55, and 45 mg/dL glucose, respectively. These results showed that pramlintide did not impair the symptom, catecholamine, and glucagon responses to insulin-induced hypoglycemia in healthy subjects.

AMYLIN IS A 37-amino acid polypeptide hormone, cosecreted with insulin by pancreatic β cells in response to nutrient intake.[1] While insulin acts to regulate glucose disappearance, amylin acts to regulate glucose appearance by slowing the rate of gastric emptying and suppressing postprandial glucagon secretion.[2-4]

Diabetes is a state of β-cell deficiency, and it presents the therapeutic challenge of replacing the glucoregulatory effects of both insulin and amylin. Clinical use of human amylin as a therapeutic agent is impractical due to solution instability and poor solubility. Pramlintide is a stable, soluble, and nonaggregating bioactive analog of human amylin, which is under clinical investigation as an adjunct therapy with insulin in patients with either type 1 or type 2 diabetes mellitus.

While prior studies have assessed the effect of pramlintide on hormonal and symptom responses to insulin-induced hypoglycemia in patients with type 1 diabetes, they did not allow for the assessment of glycemic thresholds of these phenomena. The present study was designed to further assess whether pramlintide impairs the symptom responses to insulin-induced hypoglycemia by investigating subjects during a 3-step, hyperinsulinemic, hypoglycemic clamp procedure. As hypoglycemic responses in patients with type 1 diabetes are invariably confounded by a number of factors, including disease duration, ambient glycemic control, and recent antecedent hypoglycemia,[7] healthy, nondiabetic volunteers were studied to isolate the effect of pramlintide on hypoglycemic symptoms and glucoregulatory hormone release.

MATERIALS AND METHODS

Subjects

Eighteen subjects were enrolled into the study (9 per treatment sequence) and were randomized to study medication (Table 1). Key enrollment criteria required that subjects meet the following inclusion criteria: healthy nonsmokers between 18 and 40 years old, stable body weight for 2 months prior to screening, body mass index (BMI) between 20 and 28 kg/m^2 (inclusive), fasting plasma glucose concentration between 75 and 100 mg/dL, and normal clinical laboratory tests. Additionally, females were required to have a negative pregnancy test (β-human chorionic gonadotropin [HCG]) and practice appropriate contraception. Concomitant diseases were excluded by medical history, physical examination, electrocardiogram (ECG), and clinical laboratory values. Females who were surgically sterilized by hysterectomy, or were postmenopausal, were also excluded. The study was approved by the ethics committee of Ärztekammer Nordrhein (Medical Association North Rhine, Germany) and all subjects provided written, informed consent.

Study Design

A single-center, randomized, double-blind, placebo-controlled, crossover study was used to assess the effect of pramlintide on recognition of subjective hypoglycemic symptoms and hormonal counterregulatory responses during a 3-step, hypoglycemic clamp in healthy volunteers. Subjects were enrolled into the study and were assigned a unique number from the randomization schedule on the morning of day 1 in strict chronologic order. The randomization number was associated with a treatment sequence (placebo-pramlintide or pramlintide-pla-

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Table 1. Demographic and Baseline Characteristics

Variable	Placebo/Pramlintide	Pramlintide/Placebo
n	9	9
Sex (M/F)	8/1	8/1
Race (Caucasian/other)*	100/0	100/0
Age (yr)	31 ± 8	29 ± 6
Range	20-40	24-40
Weight (kg)	81 ± 12	76 ± 13
Range	69-99	62-99
BMI (kg/m^2)	25 ± 2	24 ± 3
Range	20-28	20-28

*Race is percentage; all other data are means ± SD.

cebo) in the randomization schedule which was generated using statistical analysis software (SAS, Cary, NC).

Hypoglycemic Clamp Procedure

The study consisted of two 6-day treatment periods during which subjects were domiciled, with a minimum 3-week interval between treatment periods. Because all subjects were healthy, nondiabetic individuals, they were allowed to eat and drink ad libitum during the treatment period with the only restriction pertaining to alcohol. Additionally, on day 6 before the clamp studies, caffeine was restricted. Study medication (pramlintide 60 μg or placebo, 3 times daily) was administered subcutaneously (SC) by study personnel at approximately 8 AM, 1 PM, and 6 PM within 15 minutes prior to meals for 5 consecutive days.

On day 6, subjects received study medication (pramlintide 60 μg or placebo, SC) at approximately 8 AM within 15 minutes prior to breakfast. After consuming breakfast, subjects fasted. At approximately 3:30 PM, subjects were connected to an artificial pancreas (Biostator; MTB Medizintechnik, Ulm, Germany). At t = 0 minutes, a 60-minute baseline period began during which plasma glucose concentrations were recorded without any glucose or insulin infusion. At t = 60 minutes, a 3-step, 3-hour, hypoglycemic clamp commenced, using a primed insulin infusion[8] and a variable glucose infusion, to clamp the subject's plasma glucose concentration at 3 sequential glycemic target concentrations (70, 55, and 45 mg/dL). Each clamp step lasted for 60 minutes. A primed, continuous intravenous (IV) infusion of study medication (pramlintide, ~16 μg/h or placebo, equivalent vol/h) was also initiated at t = 60 minutes and continued until the end of the clamp experiment (t = 240 minutes).

During the baseline period and each of the 3 hypoglycemic clamp steps, a standardized, validated, visual analog scale hypoglycemic symptom questionnaire was administered at the 50- and 60-minute time points of each 60-minute clamp interval.[9,10] Subjects graded the severity of their symptoms from "None" to "Very Bad" (Fig 1). The percent score was calculated as (measurement from left of scale to mark)/(total length of scale) · 100. Blood samples were collected at 30-minute intervals throughout the clamp procedure for plasma glucose (Profil Institut für Stoffwechselforschung, GmbH, Neuss, Germany; Super GL, Ruhrtal Labortechnik, Moehnesee-Delecke, Germany), insulin (Quintiles, UK, microparticle enzyme immunoassay, Axsym, Abbott Laboratories), pramlintide (Amylin Pharmaceuticals, San Diego, CA, immunoenzymetric assay), catecholamines (Laboratoire Marcel Merieux, Lyon, France, HPLC 725 CA, TOSOH), and glucagon (Linco Research, St Charles, MO, RIA, GL-32k) measurements. Additional blood glucose measurements were obtained throughout the 3-step hypoglycemic clamp procedure for calibration of the Biostator.

At the end of the hypoglycemic clamp procedure, glucose was infused to return plasma glucose concentrations to normal range, and subjects ate a meal. Subjects remained domiciled for observation and a safety assessment was performed prior to discharge on the morning of day 7.

Statistics

Based on previous assessments, 18 subjects were judged adequate to provide the desired information about the effect of pramlintide treatment on the recognition of hypoglycemic symptoms in healthy volunteers. Subjects who withdrew prematurely were not replaced. The evaluable population (n = 17) was used for all blood glucose (Biostator), plasma glucose, insulin, pramlintide, catecholamine, and glucagon evaluations. One subject was excluded from evaluation due to insufficient pramlintide dosing as assessed by the circulating plasma pramlintide concentration profile.

All primary and secondary endpoints and safety analyses used descriptive statistics consisting of count, mean, standard deviation/standard error, median, minimum, and maximum for continuous variables and frequency and percentage for categorical variables. For summary data, missing individual data points (if there were any) were treated as missing and no imputation methods were used. Calculated parameters were based on non-missing individual data. Summaries of categorical data indicated the number of missing observations. For scheduled measurements that were repeated out of schedule, such as repeat laboratory tests, the last measurement within a scheduled time interval was used for data summaries. All analyses were performed using SAS version 6.12 on a PC Windows platform.

RESULTS

Glucose and Insulin Concentrations

Throughout the 3-step, 3-hour, hypoglycemic clamp procedure, blood glucose concentrations closely approximated the protocol-defined concentrations of 70, 55, and 45 mg/dL in both placebo- and pramlintide-treated subjects (Fig 2). There were no significant differences in the concentrations of glucose required to attain each clamp level between the pramlintide- and placebo-treated groups (data not shown). Mean plasma insulin concentrations were comparable at the 2 study visits; at the 60-minute time point for each clamp step mean insulin

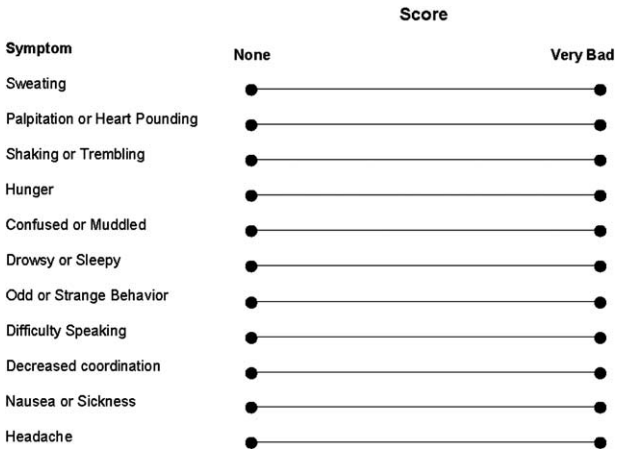

Fig 1. Autonomic/Neuroglycopenic Symptom Questionnaire. Subjects were asked to rate the intensity of their symptoms at the 50-minute and 60-minute timepoints of each clamp step by placing a mark along the line between the designations "None" and "Very Bad." The percent score was calculated as (measurement from left of scale to mark)/(total length of scale) · 100.

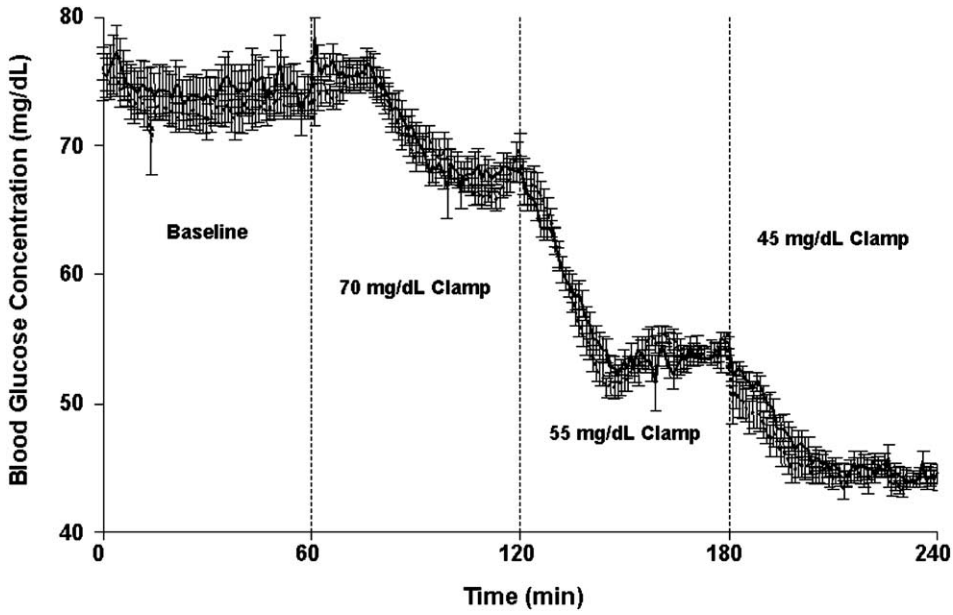

Fig 2. Plasma glucose profile of subjects during the 3-step, 3-hour, hypoglycemic clamp procedure (dashed line, placebo; solid line, pramlintide). Glucose concentrations were lowered at 60, 120, and 180 minutes to 70, 55, and 45 mg/dL, respectively. Data are means ± SEM.

concentrations were 48 ± 2.0, 50 ± 5.4, 89 ± 18.8 and 51 ± 3.2, 54 ± 5.2, 78 ± 12.8 μIU/mL (± SEM) for placebo- and pramlintide-treated subjects, respectively.

Plasma Pramlintide Concentrations

Treatment with a primed, continuous IV infusion of pramlintide (~16 μg/h) produced consistent mean plasma pramlintide concentrations of 83 ± 3.6, 82 ± 3.7, and 74 ± 3.8 pmol/L, (± SEM, 60-minute time point for each clamp step) and 76.0 ± 4.0, 87.0 ± 4.2, and 80.4 ± 4.0 pmol/L (± SEM, 30-minute time point for each clamp step) for glucose clamp concentrations of 70, 55, and 45 mg/dL, respectively. As expected, during the placebo infusion, plasma pramlintide concentrations were below the lower limit of quantitation.

Catecholamine and Glucagon Responses

Expected increases in the counterregulatory hormones epinephrine, norepinephrine, and glucagon were noted at the 2 study visits. During the hypoglycemic clamp procedure, mean epinephrine and norepinephrine responses were comparable at each clamp step (60-minute time point for each clamp step) in both the placebo and pramlintide treatment groups (Figs 3A and B). Similar responses were also observed for glucagon at each clamp step in both the placebo and pramlintide treatment periods (Fig 3C).

Autonomic/Neuroglycopenic Response

During the hypoglycemic clamp procedure, mean autonomic (Fig 4A) and neuroglycopenic (Fig 4B) symptom percent scores were comparable at each clamp step (60-minute time point for each clamp step) in both the placebo and pramlintide treatment groups. At the 45 and 55 mg/dL glucose clamps, the autonomic symptom responses were generally more pronounced than the neuroglycopenic symptom responses.

Safety

There were no serious adverse events, other significant adverse events, or withdrawals from the study. Back pain, headache, and diarrhea were the 3 most common adverse events accounting for 11% versus 0%, 17% versus 6%, and 11% versus 6% in the pramlintide versus placebo groups, respectively. Those adverse events reported were of mild-to-moderate intensity.

DISCUSSION

This study reaffirms the idea that the amylin analog pramlintide does not compromise the symptom and physiologic responses to hypoglycemia. Utilizing a 3-step, hypoglycemic clamp, subjects receiving placebo or pramlintide manifested with comparable symptoms of hypoglycemia and equivalent increments in the key counterregulatory hormones, epinephrine, norepinephrine, and glucagon.

The findings from this study align well with findings from previous studies in both rodents and human subjects. In a rodent study, amylin suppressed the glucagon response to an arginine stimulus under euglycemic hyperinsulinemic conditions, yet when plasma glucose was allowed to decrease to hypoglycemic levels, the glucagon response to hypoglycemia in rodents was robust whether or not amylin was coinfused.[4] The findings from the current study in part demonstrate a similar response in human subjects: a normal glucagon response to hypoglycemia despite coinfusion of the amylin analog pramlintide.

In previous clinical studies conducted in patients with type 1 diabetes, similar findings were again observed with some qualifications. It is well recognized that the glucagon response to hypoglycemia is deficient after 2 to 5 years of disease duration, and this likely contributes to patients' susceptibility to hypoglycemia. In this setting, the concern is not necessarily whether

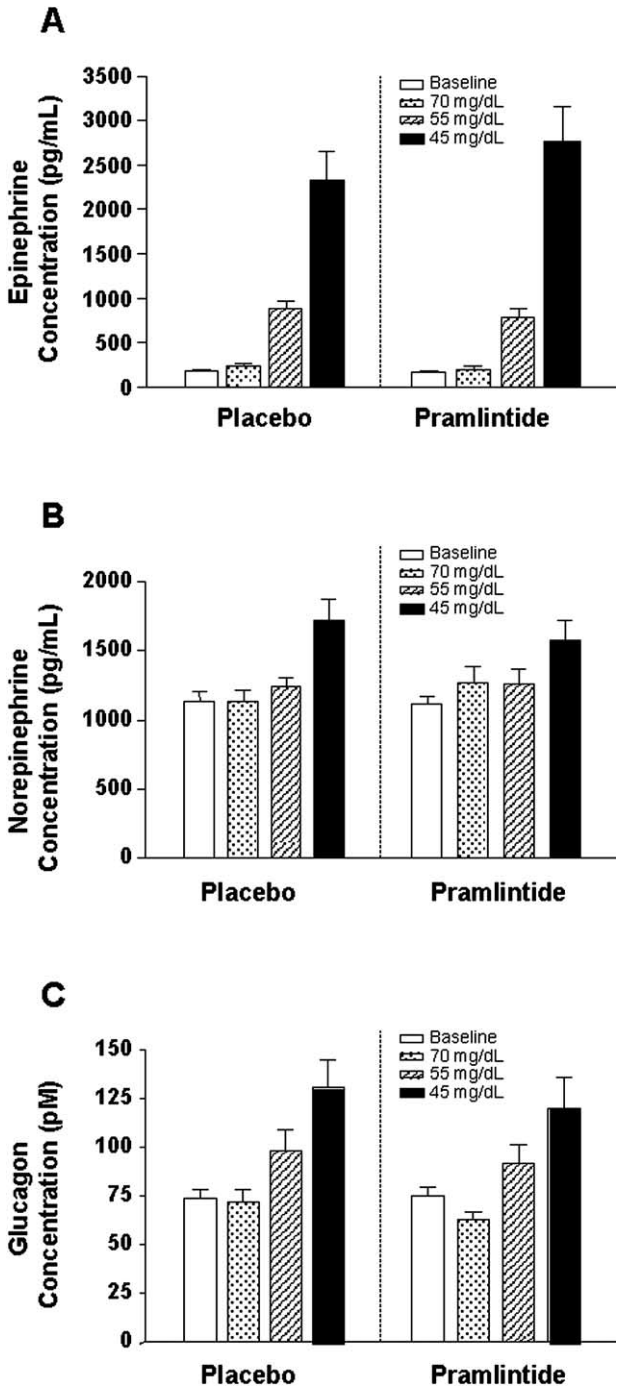

Fig 3. At the 60-minute time point of each glucose clamp step, blood samples were taken from placebo- and pramlintide-treated subjects to determine the (A) epinephrine (pg/mL) response, (B) norepinephrine (pg/mL) response, and (C) plasma glucagon (pmol/L) response. Data are means ± SEM.

or not the glucagon response is impaired by pramlintide, as it is already deficient, but whether there is a further suppression of glucagon to levels lower than that seen in the control study state. Two studies in patients with type 1 diabetes showed that glucagon levels were not suppressed by pramlintide whether administered acutely by IV infusion during a hyperinsulinemic hypoglycemia clamp[5] or administered chronically by pharmacologic subcutaneous dosing for up to 14 days prior to a hypoglycemia challenge.[11] Additional studies in patients with type 1 diabetes, undergoing a simple insulin infusion hypoglycemic challenge, showed that glucagon levels were not compromised by pramlintide.[12]

In subjects with type 1 diabetes, who have a normal glucagon response to hypoglycemia, the hypoglycemia-induced sympathoadrenal response becomes critical. The humoral and neural effects of the sympathoadrenal response on glucose counterregulation allow the normal symptom perception of a decreasing blood glucose concentration so that appropriate action may be taken. An increase in circulating catecholamines is a measure of this sympathoadrenal response. The earlier studies conducted in patients with type 1 diabetes, using either the hypoglycemic clamp technique or the insulin infusion hypoglycemic challenge, showed no suppression of catecholamine

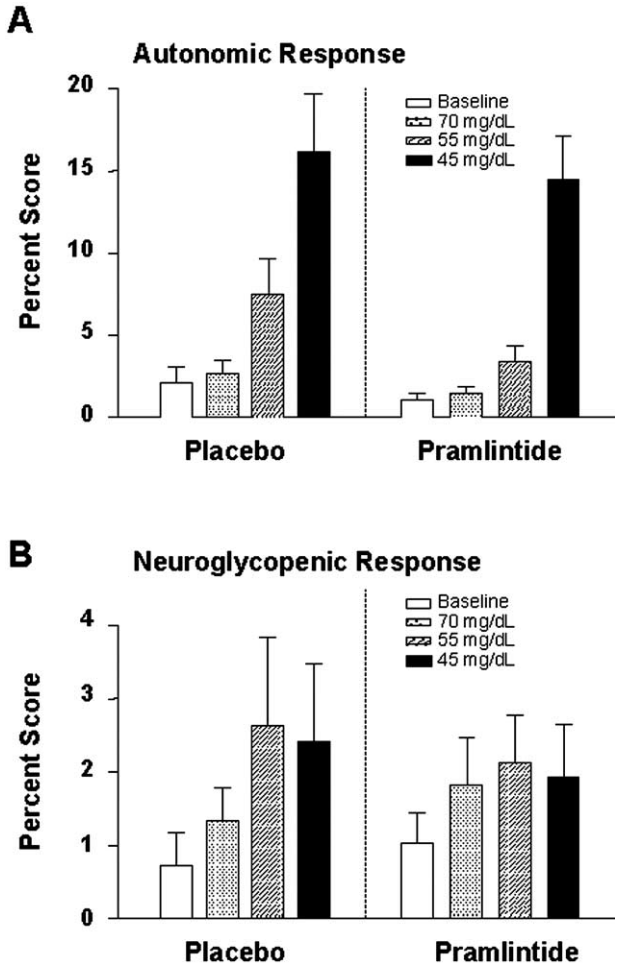

Fig 4. Average percent score for (A) autonomic and (B) neuroglycopenic responses to a standardized, validated, visual analog scale hypoglycemic symptom questionnaire administered at the 50- and 60-minute time points of each glucose clamp concentration. Data are ± SEM.

responses in the presence of pramlintide.[5] The current study reaffirms these findings during a carefully controlled 3-step hypoglycemic clamp in nondiabetic subjects. This was reflected in the average catecholamine results noted in the group as a whole, as well as in individual subject responses (data not shown). Plasma epinephrine concentrations for the pramlintide-treated group increased approximately 5-fold above baseline values at the 60-minute time point of the 55 mg/dL step and increased further by the end of the 45 mg/dL step (~16-fold). These counterregulatory responses to hypoglycaemia were at least as robust as the increases in epinephrine concentrations (~4-fold at the 60-minute time point of the 55 mg/dL step and ~12-fold at the 45 mg/dL step) seen during placebo administration.

Lastly, autonomic and neuroglycopenic symptom responses, as determined by a validated questionnaire, were also not influenced by pramlintide administration. This conclusion was drawn from close examination of autonomic symptom scores in particular. As observed in prior studies, utilizing a stepwise, hypoglycemic clamp method, autonomic symptoms occurred at a higher plasma glucose threshold than neuroglycopenic symptoms.[9] It was therefore not surprising that collective and individual neuroglycopenic symptoms were not perceived very readily by the subjects, even at the lowest hypoglycemic step (45 mg/dL). Efforts to manifest a predictable change in neuroglycopenic symptom scores are not easily achieved when one observes safety and ethical considerations. While some subjects will experience more severe symptoms at lower glucose concentrations, the margin of safety becomes unacceptable. However, autonomic symptoms are perceived more readily at a higher glycemic level, and this was reflected in both the average data for the group and the individual subject responses. Closer examination of the autonomic symptom data showed that the composite autonomic symptom score was under-representative of the perceived symptom scoring of specific autonomic symptoms. Under these controlled experimental conditions, sweating is often the earliest symptom perceived by subjects,[10,13] and indeed in this study, sweating scored higher overall than the remaining autonomic symptoms when the subjects were in an obvious hypoglycemic state (45 mg/dL). Pramlintide administration did not impair the subjects' ability to perceive sweating whether data was analyzed collectively or individually. In the minority of subjects, where sweating was not scored highly, hunger, another autonomic symptom, predominated, and this again was not influenced by pramlintide administration. As autonomic symptoms are closely coupled to the presence of the sympathoadrenal response and are manifested by an increment in circulating catecholamines, it is not surprising that autonomic symptoms would be unimpaired as the catecholamine response was also unimpaired by pramlintide.

In type 1 diabetes, the catecholamine response is impaired in many patients; therefore, these patients are particularly prone to insulin-induced hypoglycemia through ineffective glucose counterregulation and lack of hypoglycemic symptoms.[7] Factors that may impair the catecholamine and symptom response to hypoglycemia include long disease duration and recent antecedent hypoglycemia.[7,14-17] The current study is important because it lends further evidence that pramlintide does not cause an additional and independent impairment of the catecholamine and symptom response to hypoglycemia. Similar findings were also reported in the prior insulin infusion hypoglycemic challenge studies conducted in patients with type 1 diabetes where both catecholamine responses and symptom reporting were equivalent in the pramlintide and placebo treatment groups.[11]

From a methodologic standpoint, the 3-step hypoglycemic clamp utilized in this study appeared to elicit an appropriate series of counterregulatory responses in the nondiabetic subjects at both study visits. The greater relative increment in epinephrine when compared with norepinephrine is again in keeping with previous findings where a hypoglycemic stimulus in both nondiabetic subjects and patients with diabetes is characterized by a predominant adrenomedullary response and therefore a greater relative increase in epinephrine. Finally, the pramlintide infusion sustained steady state pramlintide concentrations at the high-end peak concentration of what one would expect to observe in patients with type 1 diabetes administering pharmacologic doses of pramlintide SC.

In summary, no clinically meaningful differences between pramlintide and placebo treatments were revealed in autonomic and neuroglycopenic symptom responses, in plasma catecholamine concentration responses, or in glucagon response to plasma glucose concentrations as low as 45 mg/dL in healthy volunteers. These results reaffirm that pramlintide at doses anticipated for clinical use should not impair the symptom, catecholamine, and glucagon responses to hypoglycemia in insulin-treated patients with type 1 diabetes.

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