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Evaluation of the Counter-regulatory Responses to Hypoglycemia in Patients with Type 1 Diabetes during Opiate Receptor Blockade with Naltrexone

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Abstract

Aims—Hypoglycemia is the major limiting factor in achieving optimal glycemic control in people with type 1 diabetes (T1DM), especially intensively treated patients with impaired glucose counterregulation during hypoglycemia. Naloxone, an opiate receptor blocker, has been reported to enhance the acute counterregulatory response to hypoglycemia when administered intravenously in humans. The current study was undertaken to investigate if the oral formulation of

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AUTHOR CONTRIBUTIONS:

Sarita Naik: 1) made an important contribution to the conception and design; and acquisition, analysis and interpretation of data in the study; 2) drafted and revised the manuscript critically for intellectual content; and 3) approved the final version of the submitted manuscript. **Renata Belfort-DeAguiar:** 1) made an important contribution to the conception and design; and acquisition, analysis and interpretation of data in the study; 2) revised the manuscript critically for intellectual content; and 3) approved the final version of the submitted manuscript. **Anne-Sophie Sejling,** 1) made an important contribution to the analysis and interpretation of data in the study; 2) revised the manuscript critically for intellectual content; and 3) approved the final version of the submitted manuscript. **Barbara Szepletowska:** 1) made an important contribution to the analysis and interpretation of data in the study; 2) drafted and revised the manuscript critically for intellectual content; and 3) approved the final version of the submitted manuscript. **Robert S. Sherwin:** 1) made an important contribution to the conception and design, and analysis and interpretation of data in the study; 2) revised the manuscript critically for intellectual content; and 3) approved the final version of the submitted manuscript.

CONFLICT OF INTERESTS:

Sarita Naik has no conflicts of interest associated with this manuscript. Renata Belfort-DeAguiar has no conflicts of interest associated with this manuscript. Anne-Sophie Sejling has no conflicts of interest associated with this manuscript. Barbara Szepletowska has no conflicts of interest associated with this manuscript. Robert Sherwin has no conflicts of interest associated with this manuscript.

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the long acting opiate antagonist, naltrexone, could have a similar effect, and thus might be useful therapeutically in T1DM patients with a high risk for hypoglycemia.

Materials and Methods—We performed a randomized, placebo-controlled, double-blinded, cross-over study in which 9 intensively treated subjects with T1DM underwent a 2-step euglycemic-hypoglycemic-hyperinsulinemic clamp on two separate occasions. Twelve hours and 1 hour before the clamp study the participants received 100 mg of naltrexone or placebo orally. Counterregulatory hormonal responses were assessed at baseline and during each step of the hyperinsulinemic-clamp.

Results—Glucose and insulin levels did not differ significantly between the naltrexone and placebo visits; as well as the glucose infusion rates required to keep glucose levels at target. During hypoglycemia, naltrexone, in comparison to the placebo group, induced an increase in epinephrine levels ($P=0.05$). However, no statistically significant differences in glucagon, cortisol, and growth hormone responses were observed.

Conclusion—In contrast to the intravenous opiate receptor blocker naloxone, overnight administration of the oral long acting opiate receptor blocker, naltrexone, at a clinically used dose, had a limited effect on the counterregulatory response to hypoglycemia in intensively treated subjects with T1DM.

Keywords

hypoglycemia; naltrexone; counter-regulatory hormonal response

INTRODUCTION

The Diabetes and Complications Trial (DCCT) confirmed the long-term benefits of tight glycemic control and this remains the goal therapeutically for patients with type 1 diabetes (T1DM)(1). However, intensive insulin regimens increase the risk for recurrent hypoglycemia, which commonly induces a diminished ability to recognize hypoglycemia (hypoglycemia unawareness) and an impairment in the patient's hormonal counterregulatory response, a phenomenon known as hypoglycemia-associated autonomic failure (HAAF) (2). The development of HAAF occurs in approximately 20% of patients with T1DM; and is a major limitation for the clinical implementation of intensive insulin treatment in T1DM (3, 4). Indeed, severe hypoglycemia is a significant cause of morbidity as well as mortality in such T1DM patients (5, 6). Thus, there is an urgent need to develop new, safe strategies in prevention and treatment of hypoglycemia unawareness. Recent research in this area has focused on changes in insulin preparations and insulin delivery as well as development of closed loop systems to diminish hypoglycemia risk in order to reverse or prevent the development of HAAF (7–9). However pharmacological treatments with beta2-agonists and opioid antagonists have also been tested for prevention and treatment of hypoglycemia (10–13).

Nakao et al (14) reported that plasma beta-endorphins are released in response to insulin-induced hypoglycemia. Subsequent studies found that an intravenous infusion of naloxone, an opiate receptor blocker, enhanced the counterregulatory hormone response to hypoglycemia in dogs (15) as well as in healthy non-diabetic humans and in patients with

T1DM (10). More recently, the impact of the opioid system on recurrent hypoglycemia was further investigated in healthy volunteers(16) and in patients with T1DM (17). In the later study, naloxone was reported to ameliorate HAAF by improving counterregulatory hormonal responses. Naloxone, however, can only be given subcutaneously or intravenously, limiting its therapeutic use in an outpatient setting. In contrast, naltrexone (unlike naloxone) is an oral opioid antagonist approved for the treatment of opioid and alcohol dependence(18). Thus, the aim of this study was to evaluate if the oral formulation of naltrexone, a long acting opiate antagonist, will enhance the counterregulatory response to hypoglycemia in intensively insulin-treated patients with type 1 diabetes.

MATERIALS AND METHODS

The study was a randomized, placebo-controlled, double-blinded, cross-over study. Nine intensively treated T1DM patients (7 male and 2 females) age 33 ± 4 years (Mean (SE)), duration of diabetes 17 ± 4 years, with a BMI 25 ± 1 kg/m² and a Hb1Ac of 7.0 ± 0.3 were studied on two separate occasions. Study participants came to the Hospital Research Unit (HRU) of Yale New Haven Hospital for a screening visit. They were excluded if they had any major medical disorders (liver, cardiovascular, kidney disease), diabetes complications (neuropathy, nephropathy, or retinopathy), hypertension, asthma, and cardiac arrhythmias. Participants who qualified were invited to return to the HRU for the study procedures. The study included two separate visits at least 1 week and not more than 2 months apart, for administration of naltrexone or placebo followed by a 2-step euglycemic-hypoglycemic hyperinsulinemic clamp ($2\text{mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) for 150 minutes (Fig 1). All clamp studies were performed at the same time of the day in the morning, after a 10-hour overnight fast. Participants were admitted to the HRU the night before of the clamp study where they received an overnight basal intravenous insulin (regular insulin) infusion to maintain glucose levels at a target range (100–150 mg/dL). The study was cancelled if the participants developed hypoglycemia, defined as a blood glucose level below 50 mg/dL 24 hours prior to admission to the HRU (one of the 10 initially enrolled subjects was excluded from the study based on this criteria). In the morning of the study, an intravenous plastic catheter was inserted in an antecubital vein for insulin and glucose infusion and a second intravenous catheter was placed in the contralateral arm for blood draws. Participants received a dose of 100 mg of naltrexone or placebo orally 12 hours and 1 hour before the hypoglycemic-clamp, for a total of 2 doses. To assure compliance with the study treatment, pill ingestion was observed and documented by the HRU nurses. In the following morning, 1 hour after the 2nd dose of naltrexone or placebo was given, the clamp study commenced. The naltrexone dose was calculated based on naloxone doses previously used to improve counterregulatory responses (10, 16, 17), bioequivalence (19–21), and the current FDA-approved dose for treatment of opioid addiction (less than 150 mg per day)(18). Hepatotoxicity has been reported with doses greater than 300 mg per day(22). The protocol was approved by the Yale University Institutional Review Board (Human Investigation Committee). All subjects provided written informed consent prior to enrolling in the study. This clinical trial was registered at ClinicalTrials.gov.

Hyperinsulinemic Euglycemic-Hypoglycemic clamp

Prior to the clamp study, baseline blood samples were obtained for glucose and counterregulatory hormone (epinephrine, norepinephrine, glucagon, growth hormone and cortisol) measurements. At time “zero”, an intravenous continuous insulin infusion was started at a rate of $2\text{mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and 20% dextrose was started and adjusted based on plasma glucose levels to maintain glucose levels within the euglycemic range (95–100 mg/dL) for 60 minutes. Subsequently, plasma glucose levels were allowed to fall and then maintained in the hypoglycemic range (50–55 mg/dL) for 60 minutes. Plasma insulin and counter regulatory hormone levels were obtained throughout the entire study.

Biochemical Analysis

Plasma glucose concentration was determined by the glucose oxidase method (YSI, Yellow Springs, OH). Plasma insulin and glucagon (Millipore, St. Charles, MO), growth hormone (MP Biomedical, Irvine, CA) and cortisol (Diagnostic Products Corp, Los Angeles, CA) were measured by double-antibody radioimmunoassay and plasma catecholamines were measured by high performance liquid chromatography (ESA, Chelmsford, MA).

Statistical Methods

All values represent the mean $\pm$ standard error (SE). A two-way repeated measures analysis of variance (ANOVA) was performed to identify the interaction effect of treatment over time within each group, followed by post hoc analysis. Comparisons within subjects were determined by a two-tailed Student *t* test for paired samples; and between subjects by unpaired Student *t* test for equality of means. A value of $P < 0.05$ was considered statistically significant. Graphpad Prism 4.0 software (San Diego, CA) was used for analysis.

RESULTS

Each of the intensively treated T1DM patients was studied on two separate occasions. Fasting plasma glucose levels at baseline (Naltrexone: 142 ± 6 mg/dL vs. Placebo: 148 ± 10 mg/dL; $P = \text{NS}$) as well as during the euglycemia (Naltrexone: 90 ± 4 mg/dL vs. Placebo: 101 ± 6 mg/dL; $P = \text{NS}$) and the hypoglycemia study phase (Naltrexone: 53 ± 3 mg/dL vs. Placebo: 53 ± 2 mg/dL; $P = \text{NS}$) were not significantly different during both the naltrexone and placebo sessions (Figure 2 A). Similarly, insulin concentrations at baseline and during the hypoglycemia clamp were identical between treatment groups (Figure 2 B). The glucose infusion rate (GIR) during the whole study and separately during each of the specific glucose target phases was not significantly different on both study sessions (Figure 2 C).

At baseline, prior to insulin infusion, there were no differences in the measured hormone levels (glucagon, epinephrine, norepinephrine, cortisol, and growth hormone) (Table 1). Evaluation of the effect of treatment with naltrexone vs. placebo on counterregulatory hormonal responses for glucagon, norepinephrine, cortisol, and growth hormone during hypoglycemia showed no treatment by time interaction effect in the T1DM subjects (2-way repeated measures ANOVA, $P = \text{NS}$) (Table 1 and Figure 3). However, the epinephrine response to hypoglycemia was significantly increased between the two treatment groups (2-

way repeated measures ANOVA; $P=0.05$) during the hypoglycemic phase of the hyperinsulinemic clamp. The area under the curve for epinephrine response was 2-fold higher ($P<0.05$) during the naltrexone study (Figure 3). A similar trend was observed for the GH response (Naltrexone: 2033 ± 485 vs. Placebo: 936 ± 430 ng/mL; $P=0.061$), but not the other hormones ($P=NS$).

Side Effects: no serious adverse events were noted during or immediately after these studies. Three participants in the naltrexone and one in the placebo treatment group reported feeling nauseated. One participant in the naltrexone group reported insomnia.

DISCUSSION

Release of endogenous opioids from the anterior pituitary into the peripheral circulation is a prominent response to hypoglycemia (14), but its role biologically in glucose recovery remains uncertain. In the brain, opioid receptors are present in glucose sensing regions (23) and infusion of beta-endorphin into hypothalamus has been reported to inhibit corticotropin-releasing factor secretion during hypoglycemia (24). Furthermore, it has been suggested that local release of beta-endorphins into the ventromedial hypothalamus (VMH), a key brain glucose-sensing region during hypoglycemia, is responsible for blunting the catecholamine response as well as the subsequent development of counter-regulatory failure (25). This view is supported by animal studies demonstrating a maladaptive VMH molecular response to hypoglycemia can be prevented by naloxone (26), an agent with a short half-life that is not present in an oral formulation.

This proof-of-concept study was therefore undertaken to determine the effect of naltrexone, a clinically available long-acting opioid antagonist, on hypoglycemia defense mechanisms in intensively-treated T1DM patients. We observed that pre-treatment with naltrexone, given orally prior to an acute episode of hypoglycemia, did not reduce the GIR needed to maintain glucose levels at target during a hyperinsulinemic hypoglycemic clamp and only modestly enhanced the epinephrine response to hypoglycemia. Other counterregulatory hormones, including glucagon and norepinephrine were not significantly affected. These results are in marked contrast with previous studies using intravenous naloxone, but are consistent with a recent naltrexone study reported by Moheet et al (13). In that study naltrexone given for 4 weeks did not affect the frequency of hypoglycemia, hypoglycemia symptoms score, or counterregulatory hormonal responses during a hypoglycemic-clamp in contrast to previous studies using naloxone (10, 16, 17). The discrepancy in the counterregulatory response to hypoglycemia between naltrexone and naloxone may in part be explained by differences in their mode of delivery or capacity to bind to specific opioid receptors. Beta-endorphins administered centrally in conscious dogs produce increases in circulating glucose due to a marked increase in catecholamines (27), whereas peripheral infusion has been reported to reduce glucose production, possibly through a direct effect on the liver (28). Naltrexone much like naloxone, is an antagonist of the μ -, δ -, κ -opioid receptors, however, it has the highest affinity for the μ - and κ -receptors, whereas naloxone has a higher affinity for μ - and δ -receptors (29). Naltrexone binds in a dose-dependent manner to the opioid receptors with initial blockade of the μ -receptor followed by the δ - and κ -opioid receptors respectively (30). Positron emission tomography scanning showed that 50 mg of naltrexone blocked 95%

of the μ receptors, but only 21% of the δ -receptors in the brain (31). This selective binding for specific opioid receptors may explain the disparity in results between these different pharmacological agents.

It should be noted that we cannot exclude that some of the discrepancies between prior naloxone and the current naltrexone study could have been due differences in the subject population studied (10). Our subjects had a history of frequent episodes of hypoglycemia and thus we cannot exclude that antecedent hypoglycemia episodes prior to the study visit could have affected our results. One of the limitations of this study is hypoglycemia awareness scores were not obtained at baseline in our study participants; which could have helped identify a subgroup of patients that might have benefited from naltrexone treatment. In addition, hypoglycemia symptom scores were not obtained during the clamp study and thus we were not able to determine if naltrexone could augment hypoglycemic symptom score. Finally, it is possible that the period of administration (12 hours) was insufficient to see an effect. However a different treatment strategy with a month long treatment with this same medication showed similar results (13). Both studies used similar doses of naltrexone, which are recommended for its primary treatment indication, i.e. substance abuse. It is possible that a higher dose of naltrexone might be needed, since we observed a modest increase in epinephrine with this agent during hypoglycemia. On the other hand, this possibility may be limited by its known side-effects, such as nausea and liver toxicity.

In summary, oral administration of the opioid antagonist naltrexone to T1DM patients prior to a hypoglycemic clamp did not significantly enhance their capacity to defend against hypoglycemia. However, a modest increase in plasma epinephrine occurred suggesting the potential utility of this therapeutic approach with the development of more specific opioid receptor antagonists with different pharmacological properties, and/or more potent formulations.

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Figure 1.
Hyperinsulinemic Euglycemic-Hypoglycemic Clamp Study Design.

Figure 3. Counterregulatory hormone levels during the hyperinsulinemic euglycemic-Hypoglycemic Clamp: (A) Epinephrine; (B) Norepinephrine; (C) Glucagon; (D) Cortisol; and (E) Growth

hormone. Left panel: hormone levels during the whole study session; and Right panel: area under the curve (AUC) from 0 to 150 min. Black circle naltrexone, grey square placebo. Data are the mean $\pm$ SE. * $P < 0.05$ naltrexone vs. placebo treatment using two-way repeated measures ANOVA (for a treatment by time interaction).

Table 1

Counterregulatory Hormone levels during the Hyperinsulinemic Hypoglycemic clamp study with either Naltrexone or Placebo intervention.

	NALTREXONE	PLACEBO
Glucagon (pg/mL)		
Baseline	56 ± 8	54 ± 5
Euglycemia	48 ± 6	49 ± 5
Hypoglycemia	50 ± 5	49 ± 4
Epinephrine (pg/mL)		
Baseline	43 ± 12	33 ± 9
Euglycemia	50 ± 12	37 ± 10
Hypoglycemia	111 ± 28 *	55 ± 15
Norepinephrine (pg/mL)		
Baseline	135 ± 20	158 ± 31
Euglycemia	185 ± 26	185 ± 27
Hypoglycemia	203 ± 27	193 ± 28
Cortisol (µg/dl)		
Baseline	17 ± 2	17 ± 3
Euglycemia	19 ± 1	16 ± 2
Hypoglycemia	16 ± 2	14 ± 2
Growth Hormone (ng/ml)		
Baseline	6 ± 3	4 ± 2
Euglycemia	16 ± 6	5 ± 2
Hypoglycemia	17 ± 6	10 ± 6

Data are mean ± SE. Baseline represents the average hormonal level at –20 and 0, euglycemia represents the hormone levels at the end of the euglycemia phase (60 minutes) and hypoglycemia represents the hormone levels during hypoglycemia (average 105–150 minutes) of the 2-step euglycemic-hypoglycemic hyperinsulinemic clamp.

* $P < 0.05$ vs. euglycemic-phase.