Opioid Receptor Activation Impairs Hypoglycemic Counterregulation in Humans

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Although intensive glycemic control improves outcomes in type 1 diabetes mellitus (T1DM), iatrogenic hypoglycemia limits its attainment. Recurrent and/or antecedent hypoglycemia causes blunting of protective counterregulatory responses, known as hypoglycemia-associated autonomic failure (HAAF). To determine whether and how opioid receptor activation induces HAAF in humans, 12 healthy subjects without diabetes (7 men, age 32.3 ± 2.2 years, BMI 25.1 ± 1.0 kg/m^2) participated in two study protocols in random order over two consecutive days. On day 1, subjects received two 120-min infusions of either saline or morphine (0.1 μg/kg/min), separated by a 120-min break (all euglycemic). On day 2, subjects underwent stepped hypoglycemic clamps (nadir 60 mg/dL) with evaluation of counterregulatory hormonal responses, endogenous glucose production (EGP, using 6,6-D2-glucose), and hypoglycemic symptoms. Morphine induced an ∼30% reduction in plasma epinephrine response together with reduced EGP and hypoglycemia-associated symptoms on day 2. Therefore, we report the first studies in humans demonstrating that pharmacologic opioid receptor activation induces some of the clinical and biochemical features of HAAF, thus elucidating the individual roles of various receptors involved in HAAF's development and suggesting novel pharmacologic approaches for safer intensive glycemic control in T1DM.

Intensive insulin therapy in type 1 diabetes mellitus (T1DM) has been clearly shown to reduce many diabetes-associated complications, and thus achievement of near-normal glycemia is an important management goal (1). However, despite clear clinical benefits, intensive therapy is associated with an increased risk of iatrogenic hypoglycemia, with a threefold increase in severe hypoglycemia reported in the intensively treated group in the Diabetes Control and Complications Trial (DCCT) (1) and even higher rates reported more recently among patients with T1DM by the U.K. Hypoglycemia Study Group (2). Despite medical advances in diabetes management, the problem of iatrogenic hypoglycemia has not been ameliorated (3) and remains both a clinical challenge and a costly public health problem. Indeed, there are an estimated nearly 100,000 emergency department visits and 30,000 hospital admissions for insulin-related hypoglycemia yearly in the U.S. alone (4). Furthermore, hypoglycemia per se causes morbidity and may even be fatal, with 6–10% of deaths in patients with T1DM attributed directly to hypoglycemic events (5).

Patients with T1DM are at particular risk of frequent hypoglycemia due to exogenous insulin treatment because they demonstrate blunted hormonal counterregulatory responses to hypoglycemia (6). In addition, it has been well established in both subjects without diabetes (7) and those with T1DM (6,8) that stressors such as recurrent hypoglycemia or exercise lead to blunting of protective glucagon and sympathoadrenomedullary counterregulatory responses as well as deterioration of hypoglycemia awareness and recovery, conditions known as hypoglycemia-associated autonomic failure (HAAF) and exercise-associated autonomic failure (EAAF), respectively (6).

Although the exact mechanisms underlying the development of HAAF and EAAF have not been fully elucidated, central nervous system (CNS) signals mediating the counterregulatory response have been implicated in its pathogenesis (9). Robust data point to a key role of the endogenous

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opioid system in the development of HAAF and EAAF. Many kinds of stressors, including hypoglycemia and exercise, precipitate release of endogenous opioids, such as β-endorphin, that can mediate autonomic and sympathoadrenomedullary responses in humans and animals (10).

In fact, it has been proposed that HAAF and EAAF may represent a form of stress habituation to recurrent hypoglycemia (11), possibly as a defensive adaptation, particularly since most features of HAAF are reversible after a 2–3-week period of scrupulous hypoglycemia avoidance (12). One can speculate that this may have been an evolutionary protective mechanism during times of famine or prolonged exercise. Furthermore, opioid receptor blockade with naloxone infusion during experimental hypoglycemia prevents the development of HAAF in subjects without diabetes and ameliorates HAAF in subjects with T1DM (13–15). Similarly, naloxone infusion during antecedent exercise prevents development of EAAF in humans without diabetes (16), and the magnitude of β-endorphin release during exercise is inversely correlated with catecholamine release during subsequent hypoglycemia (17). Taken together, these data suggest that central release of endogenous opioids during hypoglycemia or exercise may suppress the counterregulatory response to hypoglycemia. However, the fact that naloxone has therapeutic effects in HAAF and EAAF does not provide conclusive evidence that opioid action underlies these conditions. Thus, detailed mechanistic studies clarifying the importance of the opioidergic system in the development of HAAF are warranted. We therefore examined whether pharmacologic activation of μ-opioid receptors with morphine over a time course comparable to a bout of hypoglycemia or exercise would precipitate HAAF in humans.

RESEARCH DESIGN AND METHODS

Study Subjects

We studied 12 healthy volunteers without diabetes (7 men, 5 women, age 32.3 ± 2.2 years, BMI 25.1 ± 1.0 kg/m^2, HbA_{1c} 5.4 ± 0.1%). All were in good health and taking no medications and had no history of hypoglycemia or family history of diabetes. Each subject participated in two different sets of studies, in random order, with an interval of at least 5 weeks between studies. All studies were performed after an overnight fast. Each set of studies consisted of two consecutive days. Day 1 in each set consisted of two 120-min infusions of either normal saline (control) or morphine (0.1 μg/kg/min), separated by a 120-min break during which subjects received a small snack (15 g of carbohydrate). Subjects remained euglycemic throughout day 1 in all studies. Continuous cardiac monitoring, respiratory monitoring, and capnography to monitor the partial pressure of expired carbon dioxide were used throughout all day 1 studies (Waveline EZ Monitor with Sidestream CO2 Sensor; DRE Med, Louisville, KY). Day 2 was identical in all studies and consisted of a hyperinsulinemic stepped hypoglycemic clamp, with quantification of hormonal responses and glucose kinetics. The research protocol was approved by the Institutional Review Board of the Albert Einstein College of Medicine, and informed written consent was obtained in accordance with the Institutional Review Board policy. Subjects were admitted to the Clinical Research Center for each experiment.

Day 1: Morphine Versus Normal Saline Infusion Under Normoglycemic Conditions

At 0800 h on the study day, all subjects had two indwelling catheters inserted. One was placed in an antecubital vein for infusions, and the second was placed in a retrograde fashion in a distal hand vein of the contralateral forearm for blood sampling. To obtain arterialized venous blood samples, this hand was maintained at 65°C in a thermoregulated sleeve. As depicted in Fig. 1*A*, at t = 0 min, a constant infusion of either normal saline (control) or morphine (0.1 μg/kg/min) was initiated. Subjects' heart rate, respiratory rate, electrocardiogram tracing, and CO_2 levels

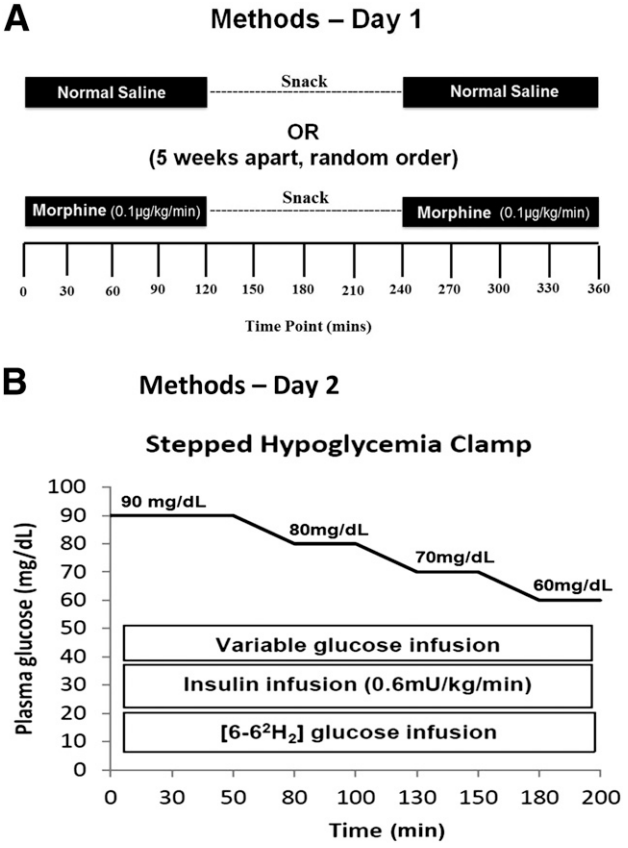

Figure 1—Study protocol. *A*: On day 1, each subject received a 2-h infusion of either normal saline or morphine (to simulate a placebo or hypoglycemic event, respectively). Infusions were discontinued for a 2-h interval during which time the subjects received a snack, and then the same infusion was repeated. Each subject was randomly assigned to receive both infusions, separated by at least 5 weeks. *B*: On day 2, each patient underwent a stepped hypoglycemic clamp study. This was identical in all protocols. Insulin was infused at a constant rate for the entire study. Plasma glucose concentrations were clamped for 50-min intervals at each target level: 90, 80, 70, and 60 mg/dL. Symptoms of hypoglycemia were measured at each step.

were monitored continuously throughout the study. Blood samples were collected at 30-min intervals for measures of plasma glucose and counterregulatory hormones. At t = 120 min, infusions were discontinued and subjects received a 15-g carbohydrate snack, consisting of a small piece of fruit. Subjects then rested quietly for 120 min, and at t = 240 min, the experimental conditions were resumed, with subjects assigned to the same conditions as during the first 120-min interval. At t = 360 min, infusions were discontinued, a meal was provided, and subjects were discharged.

Day 2: Stepped Hypoglycemic Clamps

The study conducted on day 2 was identical in all protocols, and a schematic depiction of the methods used is shown in Fig. 1*B*. At 0800 h, subjects had two indwelling catheters inserted. At $t = -120$ min, a primed continuous infusion of 6,6-D2-glucose (D2G) tracer was initiated (200 mg/m^2 bolus followed by 3.9 mg/min for the entire study period) to measure glucose fluxes. At t = 0 min, a primed continuous infusion of insulin was initiated at a rate of 1.0 μU/kg/min for the first 10 min and thereafter was continued at 0.6 μU/kg/min throughout the study. At t = 10 min, a variable infusion of 20% dextrose was also begun to maintain the plasma glucose concentration at 90 mg/dL for 50 min. The specific activity of infused dextrose was kept equivalent to plasma glucose specific activity by addition of D2G to the infusate. At t = 50 min, and every 50 min thereafter, the plasma glucose concentration was decreased by decrements of 10 mg/dL for 50 min by reducing the dextrose infusion rate accordingly. Plasma glucose was clamped at the desired range according to plasma glucose measured at 5-min intervals with targets of 90, 80, 70, and 60 mg/dL. Blood samples were obtained for determinations of plasma insulin, C-peptide, glucagon, epinephrine, norepinephrine, and cortisol, as well as for glucose turnover. Symptoms of hypoglycemia were measured at each glucose step using the Edinburgh Hypoglycemia Score (18). At t = 200 min, all infusions were discontinued, a meal was provided, and plasma glucose was monitored for at least 1 h to ensure restoration of euglycemia prior to discharge.

Analytical Methods

Plasma glucose was measured with a Beckman glucose analyzer (Beckman Coulter, Fullerton, CA), using the glucose oxidase method. Measurements of plasma insulin, C-peptide, glucagon, and cortisol concentrations were measured by radioimmunoassay in the Diabetes Research Center Hormone Assay Core, as previously reported (19). D2G concentrations were measured by gas chromatography–mass spectrometry, as previously described (20). Plasma epinephrine and norepinephrine levels were determined using high-performance liquid chromatography (HPLC; Quest Diagnostics, Chantilly, VA). Additional confirmatory plasma epinephrine concentrations were measured by the Hormone Assay and Analytical Services Core of Vanderbilt University Medical Center using HPLC.

Analysis

The data are presented as the mean ± SEM. The Steele equation was used for calculation of glucose turnover (21). Values for endogenous glucose production (EGP) and R_d, obtained at 10-min intervals, were averaged over the final 30 min of each glucose step for each individual subject. The glycemic threshold for activation of a particular hormone was calculated as the glycemic level at which there was an increase of more than two SD values above basal concentration. Statistical analyses were performed using repeated-measures ANOVA to compare successive time points within studies, and Student t tests were used when comparisons between the two study conditions (morphine vs. saline) were examined. A value of $P < 0.05$ was considered significant.

RESULTS

Day 1: Morphine and Saline Infusions

As shown in Supplementary Table 1*A*, plasma concentrations of glucose, cortisol, epinephrine, and norepinephrine were measured every 30 min and are presented as averages over hourly intervals throughout infusions of morphine and saline (0–120 min and 240–360 min). As shown in Supplementary Table 1*B*, plasma concentrations of glucagon and insulin were measured every 120 min and are presented at times 0, 120, and 360 min, i.e., before and after infusions of morphine and saline. There were no significant differences in hormone concentrations between the saline and morphine infusions on day 1. This indicates that the morphine infusion did not induce hypoglycemia or a hypoglycemia-like hormonal profile.

Day 2: Stepped Hypoglycemic Clamp Studies

Plasma Glucose Concentrations

Plasma glucose concentrations during the hyperinsulinemic stepped hypoglycemic clamps on day 2 are shown in Fig. 2. Target plasma glucose levels were achieved in both groups, with no significant differences between the studies.

Plasma Insulin and C-Peptide Concentrations

Plasma insulin concentrations (Fig. 3*A*) were comparable in all studies at baseline, averaging 10.2 ± 0.1 μU/mL in the control studies and 7.8 ± 0.9 μU/mL in the morphine studies (P = NS). Plasma insulin concentrations were also nearly identical in both groups throughout the hypoglycemic clamps, averaging 38.3 ± 1.0 μU/mL in the control studies and 38.7 ± 0.7 μU/mL in the morphine studies (P = NS). Similarly, plasma C-peptide concentrations (Fig. 3*B*) were nearly identical between groups at baseline, averaging 1.0 ± 0.1 ng/mL in the control studies and 0.9 ± 0.1 ng/mL in the morphine studies, and during the hypoglycemic nadir of the clamp, averaging 0.2 ± 0.0 ng/mL in both study groups (P = NS for both comparisons).

Plasma Epinephrine and Norepinephrine Concentrations

Plasma epinephrine concentrations (Fig. 4*A*) were comparable in all studies during the 90 and 80 mg/dL glucose steps. Further reduction in plasma glucose levels to 70 mg/dL

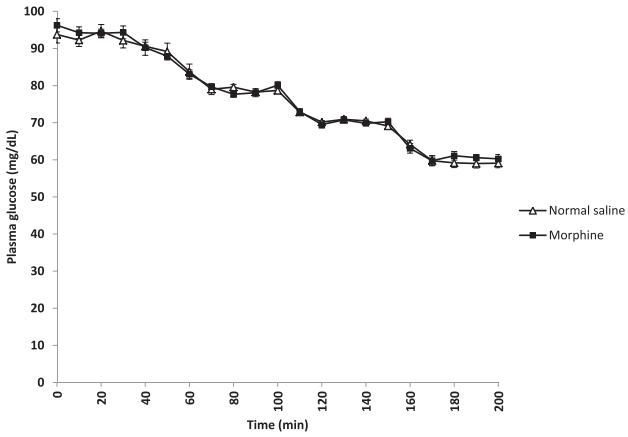

Figure 2—Plasma glucose concentrations during the stepped hypoglycemic clamp (day 2). Target plasma glucose concentrations were achieved in both groups, with no significant differences between the studies.

induced a rise in plasma epinephrine levels in both groups, averaging 133.9 ± 2.7 pg/mL in the control studies and 105.0 ± 7.0 pg/mL in the morphine studies. Although the average epinephrine concentrations were higher in the control studies at the 70 mg/dL glucose step, this was not a statistically significant difference. However, although achievement of the hypoglycemic nadir of 60 mg/dL was associated with further increases in plasma epinephrine concentrations in both study groups, significantly lower average plasma epinephrine concentrations were demonstrated in the morphine studies compared with control studies. The average plasma epinephrine concentration at the 60 mg/dL glucose step was 419.4 ± 20.4 pg/mL in the control studies and 292.5 ± 15.7 pg/mL in the morphine studies, representing a 30.3% reduction in plasma epinephrine concentration in the morphine study group (P = 0.02).

Plasma norepinephrine concentrations (Fig. 4*B*) were similar between groups at the 90 and 80 mg/dL glucose steps. Although plasma norepinephrine concentrations increased in both study groups as plasma glucose levels were reduced to 70 and 60 mg/dL, there were no statistically significant differences between the two study groups, with concentrations averaging 258.6 ± 9.8 pg/mL in the control studies and 249.5 ± 10.4 pg/mL in the morphine studies at the 60 mg/dL glucose step (P = NS).

To determine to what extent opioid receptor activation is responsible for the etiology of HAAF, the peak epinephrine responses to morphine were compared with the peak epinephrine responses to three episodes of hypoglycemia per se in an additional n = 6 healthy control subjects without diabetes (6 men, age 45.0 ± 7.1 years, BMI 25.6 ± 2.8 kg/m^2), following a study design previously reported to induce hypoglycemia-induced elevations in β-endorphins and HAAF (22). On day 1, the subjects underwent two 2-h episodes of hyperinsulinemic (~1.5 mU/kg · min) hypoglycemic (target glucose 54 mg/dL) clamp studies, separated by a 2-h break with a small snack. This was followed by a third, comparable hypoglycemic episode on day 2. Respective peak epinephrine levels were 1,005 ± 292 pg/mL for the first clamp, 859 ± 226 pg/mL for the second clamp, and 542 ± 220 pg/mL for the third clamp, demonstrating a significant reduction in peak epinephrine concentrations during the third versus the first hypoglycemic clamp (P = 0.039). Therefore, whereas morphine administration on day 1 caused an ~30% reduction in epinephrine concentrations during mild hypoglycemia on day 2, two episodes of moderate hypoglycemia on day 1 caused an ~45% decrease in epinephrine concentrations during moderate hypoglycemia on day 2. Collectively, these studies suggest that opioid receptors play a significant role in the development of HAAF. However, opioid receptor activation likely does not explain the entire phenomenon, and it is also possible that pharmacologic effects of morphine have contributed to the current findings.

Other Counterregulatory Hormones

Plasma glucagon concentrations (Fig. 4*C*) were nearly identical at baseline and increased more steeply in the initial phase of hypoglycemia (80 mg/dL) in the saline control studies relative to the morphine studies (31.7 ± 5.9 vs. 22.2+2.9 pg/mL, respectively; P = 0.029) but did not differ during subsequent hypoglycemic steps between the control and morphine studies. Plasma cortisol (Fig. 4*D*) concentrations were nearly identical at baseline and increased to a similar degree throughout the hypoglycemic clamp in both the control and morphine studies. Plasma growth hormone concentrations trended lower throughout the hypoglycemic steps of hypoglycemia in the morphine studies, particularly at the 70 mg/dL step (P = 0.097), but did not reach statistical significance (Fig. 4*E*).

EGP

EGP results are shown in Fig. 5*A*. Although rates of EGP were noted to be slightly lower in the morphine group compared with the control group at all glucose steps, these differences only reached statistical significance at the 80 mg/dL glucose step (P = 0.04). Since it was necessary to infuse exogenous glucose throughout the study in order to maintain the target glucose concentrations, this may have masked the true differences in EGP, which only reached statistical significance at the 80 mg/dL step. R_d results are depicted in Fig. 5*B*, with similar rates of glucose uptake demonstrated at each glucose step for both study groups.

Glucose Infusion Rates

Glucose infusion rates are depicted in Fig. 5*C*. There were no significant differences in glucose infusion rates during the 90 and 80 mg/dL glucose steps between the two study groups (1.6 ± 0.4 vs. 1.4 ± 0.4 mg/kg/min at 90 mg/dL and 2.1 ± 0.1 vs. 2.2 ± 0.2 mg/kg/min at 80 mg/dL for normal saline and morphine, respectively). During the 70 and 60 mg/dL glucose steps, higher glucose infusion

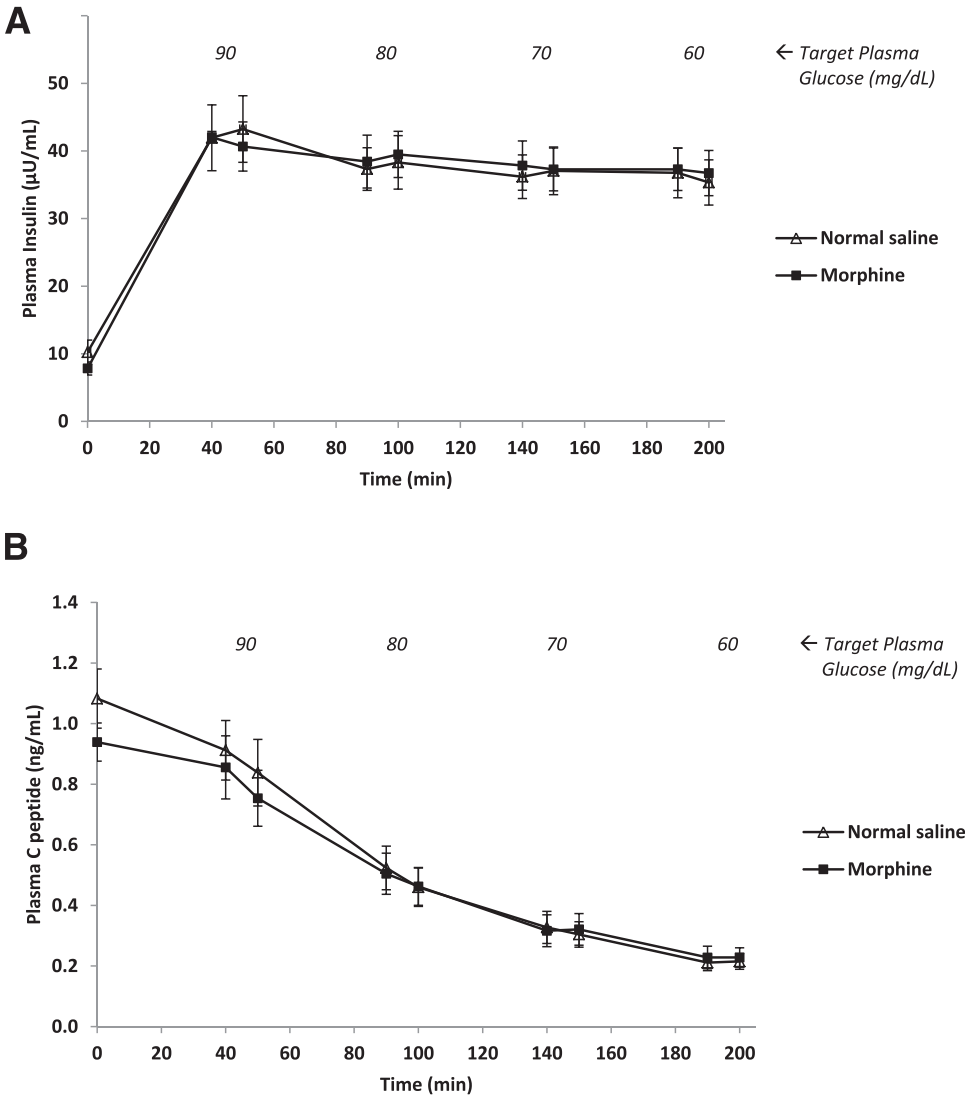

Figure 3—Plasma insulin and C-peptide concentrations. Plasma insulin (*A*) and C-peptide (*B*) concentrations were nearly identical in both groups at each target glucose level throughout the study. Average values are shown.

rates were required to maintain target plasma glucose levels in the morphine studies when compared with the normal saline studies: the mean glucose infusion rate was 1.8 ± 0.2 vs. 2.1 ± 0.2 mg/kg/min at 70 mg/dL and 0.4 ± 0.3 vs. 0.8 ± 0.2 mg/kg/min at 60 mg/dL for normal saline and morphine, respectively ($P < 0.01$ for both glucose steps).

Hypoglycemic Symptom Score

Hypoglycemic symptoms are shown as the number of symptoms reported at each glucose step (Fig. 6). Eleven symptoms were evaluated, and subjects reported significantly fewer symptoms after receiving morphine compared with control studies ($P = 0.030$ at the 60 mg/dL step, and a progressive downward trend at 80 and 70 mg/dL, respectively). Reports of hunger ($P = 0.035$) and headache ($P = 0.015$, both at 60 mg/dL) were particularly reduced when subjects had received morphine the day before completing the hypoglycemic clamp.

DISCUSSION

Despite many recent therapeutic advancements in the management of T1DM, including the development of insulin analogs, insulin pumps, and continuous glucose monitoring, maintaining near-normal glycemia remains an elusive goal for most patients, in large part owing to the risk of hypoglycemia (2). Patients with T1DM are susceptible to hypoglycemia due to defective counterregulatory responses characterized by the following: *1*) deficient glucagon release during impending/early hypoglycemia; *2*) HAAF and EAAF that blunt the sympathoadrenal responses to hypoglycemia after repeated episodes or exercise as well as diminishing other counterregulatory responses; and *3*) hypoglycemia unawareness, lowering the threshold for symptoms that trigger behavioral responses (e.g., eating). Thus, the risk of hypoglycemia in T1DM impedes the use of ideal insulin treatment and leads to suboptimal glycemic control (3).

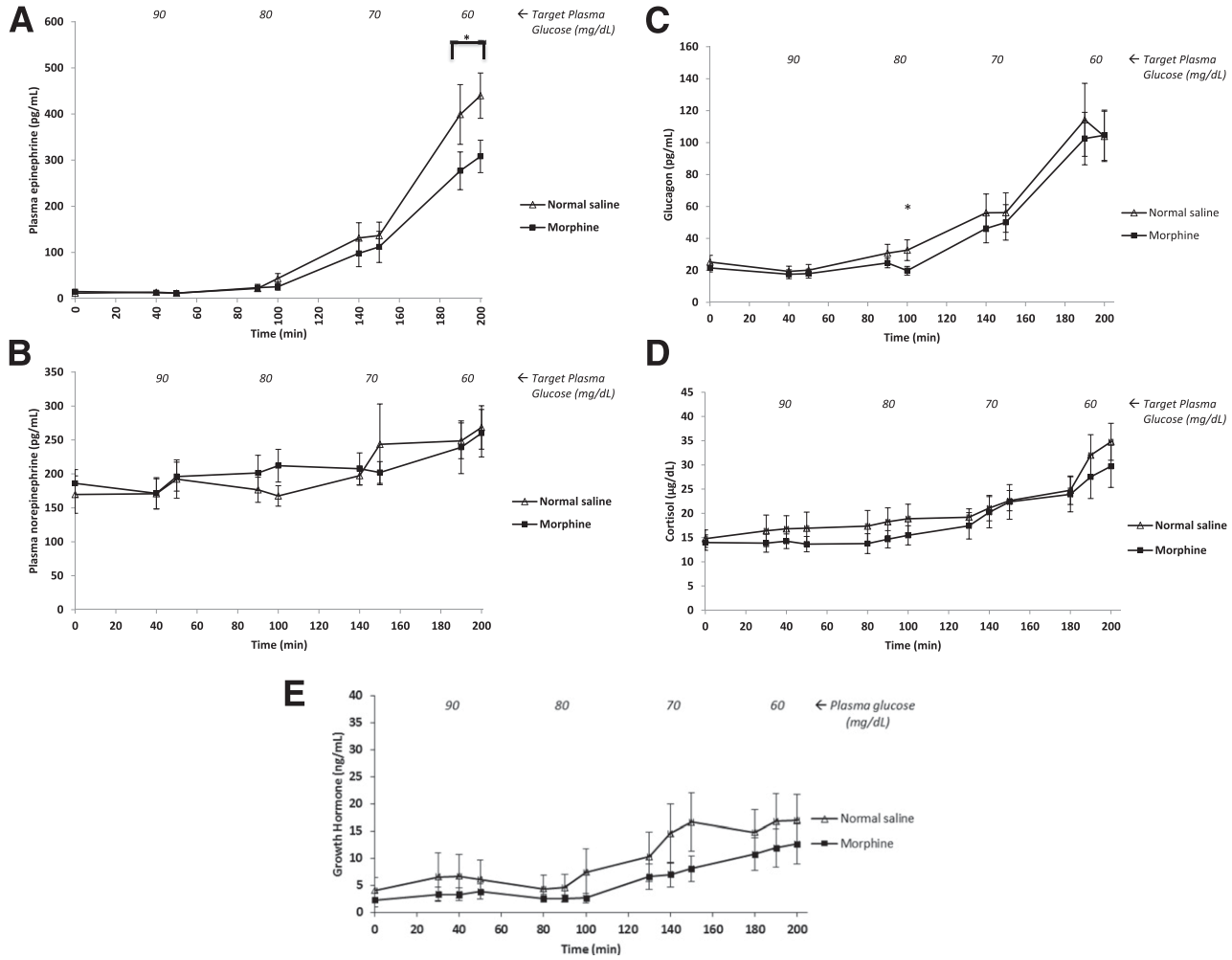

Figure 4—Plasma counterregulatory hormone concentrations. *A*: Plasma epinephrine concentrations were comparable in both groups during the 90 and 80 mg/dL glucose steps. At the hypoglycemic nadir of 60 mg/dL, there was a 30.3% reduction in epinephrine levels in the morphine study group compared with control subjects ($P = 0.02$). Plasma norepinephrine (*B*) and cortisol (*D*) concentrations were similar in both groups without any significant differences. Plasma glucagon (*C*) concentrations were significantly lower in the morphine group, but only at the 80 mg/dL glucose step. Plasma growth hormone (*E*) concentrations trended lower in the all hypoglycemic steps of the morphine studies, particularly at the 70 mg/dL step ($P = 0.097$), but did not reach statistical significance. Average values are shown. *$P < 0.05$.

We therefore designed these studies to better understand the physiologic or maladaptive mechanisms whereby HAAF develops, specifically through the activation of opioid receptors. These studies provide data that antecedent morphine infusion can reproduce some of the key features of HAAF and EAAF in humans without diabetes. The morphine infusion rate was selected based upon the relative potency of morphine and β-endorphin for the opioid receptor (23), observed relationships between plasma morphine levels and intravenous infusion rates (24), and plasma β-endorphin concentrations observed during hypoglycemia in humans (22,25). Epinephrine responses at the lowest level of hypoglycemia (60 mg/dL) were blunted in healthy subjects who had received low-dose morphine infusions the day before the hypoglycemic clamp, compared with paired hypoglycemic clamps performed the day after normal saline infusion. Similarly, the glucose infusion rates required to maintain blood glucose levels at the target level during the 70 and 60 mg/dL glucose steps were higher after subjects had received morphine the day before the clamp.

Assessment of hypoglycemic symptoms also revealed fewer symptoms at 60 mg/dL hypoglycemia on the day after morphine infusion. Taken together, these data support the ability of μ-opioid receptor activation with morphine to reproduce some key biochemical and clinical features of HAAF in humans without diabetes, and represents an important model through which mechanisms of autonomic failure may be studied in humans without inducing repeated episodes of hypoglycemia per se. Importantly, there were changes in EGP and glucose infusion rate that preceded the changes in hormone levels, suggesting that direct brain sensing of glucose regulates glucose production. This is consistent with our previous work indicating that activation of central K_{ATP} channels directly regulates glucose production in humans (26).

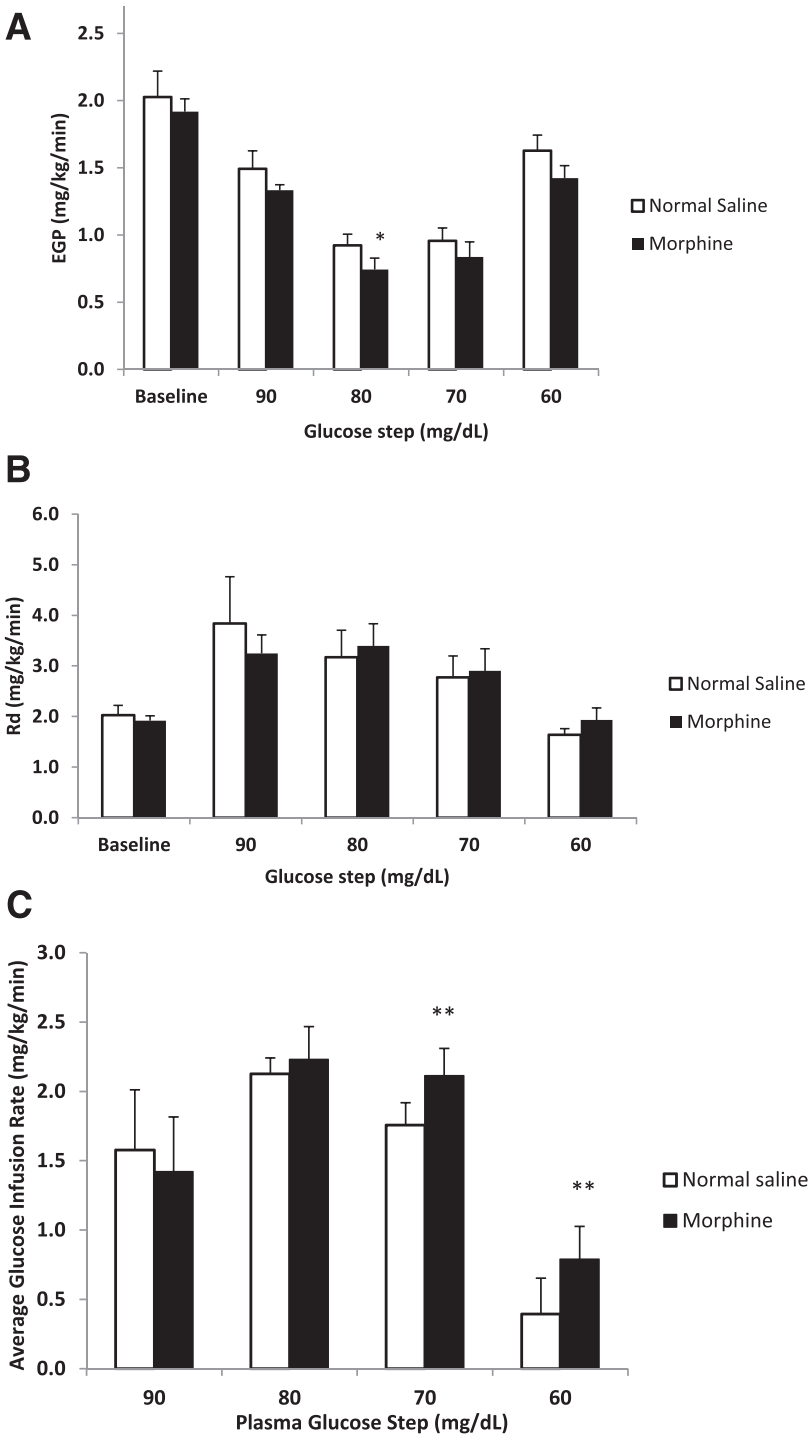

Figure 5—EGP and glucose infusion rates. *A*: EGP rates trended lower at every glucose step in the morphine studies, and these differences reached statistical significance at the 80 mg/dL glucose step ($P = 0.04$). *B*: Both groups demonstrated similar rates of glucose uptake, as quantified by R_d. *C*: Glucose infusion rates were similar during the 90 and 80 mg/dL glucose steps. During the 70 and 60 mg/dL glucose steps, higher glucose infusion rates were required to maintain target plasma glucose levels in the morphine studies when compared with the normal saline control subjects ($P < 0.01$ for both steps). *$P < 0.05$, **$P < 0.01$.

Furthermore, opioid receptor activation has allowed us to mimic stressors known to induce HAAF (i.e., hypoglycemia and exercise) while excluding the majority of factors associated with such stressors. Different mechanisms are likely involved in the regulation of catecholamine and glucagon release in response to hypoglycemia (27). Endogenous opioids are secreted by the proopiomelanocortin neurons of the pituitary gland (28) in response to a variety of stressors, including hypoglycemia and exercise (10,29,30). CNS signals that mediate the response to hypoglycemia may be of major

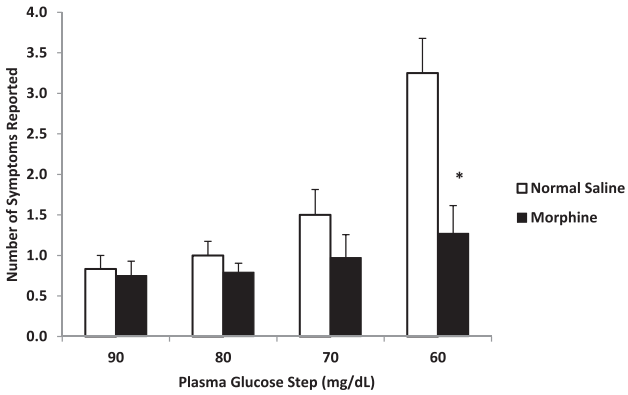

Figure 6—Hypoglycemia symptoms score. Using the Edinburgh Hypoglycemia Score, 11 symptoms of hypoglycemia were evaluated at each glucose step. During hypoglycemia the day after morphine infusion, subjects reported fewer symptoms of hypoglycemia, which reached statistical significance at the 60 mg/dL glucose step ($P = 0.03$). *$P < 0.05$.

importance in glucose counterregulation. In the CNS, opioids likely contribute to the development of HAAF via activation of opioid receptors localized to areas in the thalamus and hypothalamus responsible for glucose sensing, including the ventromedial hypothalamus, arcuate nucleus, and dorsal medial thalamus (31,32). Administration of an endogenous opioid (β-endorphin) directly into the rat brain was shown to inhibit hypothalamic responses to hypoglycemia (33). In parallel, accumulating evidence suggests that endogenous opioids produced peripherally by the adrenal medulla may lead to glucose lowering in streptozotocin-induced diabetic rats, both by increasing glucose uptake and decreasing hepatic gluconeogenesis (34,35). Finally, various studies have demonstrated that β-endorphin can modulate glucose homeostasis by its action on insulin release (36–38). In vitro, β-endorphin (which primarily targets μ-opioid receptors) inhibits insulin release from isolated islets (39), and in vivo, β-endorphin also attenuates insulin release when administered by intravenous infusion (40).

The glucose-lowering effects of β-endorphin in a T1DM–like diabetic rat model are due to an increase in GLUT4 gene expression, leading to higher glucose utilization and decreased PEPCK gene expression, leading to a decline of hepatic gluconeogenesis (41,42). Furthermore, it has recently been shown that β-endorphin release from the adrenal gland is activated by α1-adrenoreceptor stimulation (35); phenylephrine stimulation caused an increase in β-endorphin concentrations, whereas α-antagonist administration resulted in a decrease in β-endorphin levels (35,41). Endogenous opioids, in turn, induce suppression of catecholamine release from the adrenal gland, suggesting secretory negative feedback between adrenal catecholamine release and opioid secretion (43–45). Importantly, this opioid effect on the adrenal medulla is reversed in vivo with naloxone administration (46). Taken together, these data suggest that modulation of the counterregulatory response to hypoglycemia occurs both centrally and peripherally and that the opioid system plays a pivotal role in both locations.

Of note, these studies in human subjects were unable to determine the exact location(s) at which morphine acts in order to modulate hypoglycemia counterregulation. Furthermore, although morphine interacts predominantly with the μ-opioid receptor, it also may act as a κ-opioid and δ-opioid receptor agonist. Thus, our results provide new insight into the role of the opioidergic system in the physiologic and clinical response to hypoglycemia in humans.

Previous work in human subjects has demonstrated that opioid receptor blockade with naloxone results in modulation of HAAF/EAAF (14–17). As this does not provide direct evidence that opioid action underlies HAAF, the current mechanistic studies elucidate a role for the opioidergic system as a target for therapy in HAAF. However, given that only some features of HAAF were recapitulated in healthy humans using opioid receptor activation, it is clear that other important pathways are involved in the development of the full spectrum of HAAF's biochemical and clinical elements. Recent data show that adrenergic receptor blockade also prevents antecedent hypoglycemia's ability to attenuate the sympathoadrenal response to subsequent hypoglycemia (47). This is of particular interest since opioidergic and adrenergic receptors show close functional interactions. Both morphine and norepinephrine induce major inhibitory effects in brain neurons and peripherally by activating G protein–coupled receptors (48). Additionally, heterodimerization of these receptors may activate common signal transduction pathways or confer them with new functional properties that are different from the original receptors (47,49,50). Thus, isolating the role of the opioidergic system in HAAF should lay the foundation for further physiologic studies examining the other key receptors involved and their interactions in the development of HAAF.

Although experimental HAAF has been shown to be prevented in healthy humans and improved in subjects with T1DM by acute administration of intravenous naloxone during antecedent hypoglycemia (13–15), long-term administration of opioid receptor blockade and its effects on hypoglycemia counterregulation are still under investigation. Intriguingly, a recent pilot study in subjects with T1DM showed no effect of short-term oral naltrexone treatment on hypoglycemic symptoms or counterregulatory responses (51). At chronic low doses, naltrexone may have anti-inflammatory effects and result in an increase in opiate binding sites and thus supersensitivity to opioid agonists (52). It is also possible that differences in opioid responses or function secondary to long-standing T1DM may explain why opioid activation induced features of HAAF in healthy individuals in our studies, and long-term opioid antagonism did not reverse HAAF in patients with T1DM and impaired hypoglycemia awareness. This underscores the significance of the current studies of opioid agonists to clarify the opioid receptor's specific contributions in HAAF. Larger studies of greater duration or clinical studies in which oral naltrexone is

given acutely at the time of hypoglycemia will need to be performed to clarify the clinical role of opioid receptor antagonists in HAAF and to tailor effective therapies for HAAF.

It is intriguing to consider the evolutionary pressures that might have promoted the development of HAAF in humans, and what teleologic advantage(s) it might confer. HAAF could have provided an adaptive mechanism of survival during times of famine or prolonged exercise to minimize the intense energy demands of mounting a full counterregulatory response to every drop in blood glucose level. Intriguingly, it has been shown that in patients with diabetes, treated with insulin, glucose uptake within the brain is increased during periods of hypoglycemia. Maintenance of CNS glucose concentrations may prevent systemic counterregulatory responses to hypoglycemia, which may be considered a physiologically useful adaptation to preserve brain function in the presence of episodic hypoglycemia (53).

Thus, we report the first studies in humans demonstrating that pharmacologic opioid receptor activation can experimentally recapitulate some features of HAAF, without using stressors such as hypoglycemia or exercise to induce HAAF. These studies provide a model for studying HAAF in humans and offer a key step in elucidating the individual roles of various receptors in its development. A full understanding of the physiologic basis of HAAF is crucial to tailor appropriate therapies for patients with recurrent hypoglycemia.

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