Opioid Receptor Blockade Prevents Exercise-Associated Autonomic Failure in Humans

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Hypoglycemia and exercise both induce the release of β-endorphin, which plays an important role in the modulation of the autonomic response during subsequent events. Because opioid receptor (OR) blockade during antecedent hypoglycemia has been shown to prevent hypoglycemia-associated autonomic failure, we hypothesized that OR blockade during exercise would prevent exercise-associated autonomic failure (EAAF). We studied 8 healthy subjects on 2 consecutive days, each of whom participated in three different studies in random order. The protocol on day 1 involved one of the following: *1*) two 90-min hyperinsulinemic-euglycemic clamps plus naloxone infusion (control); *2*) two 90-min hyperinsulinemic-euglycemic clamps with exercise at 60% $V_{O_{2max}}$, plus naloxone infusion (N+); or *3*) same protocol as in the N+ group, but with saline infusion only (N−). On day 2, all were studied with stepped hyperinsulinemic-hypoglycemic clamps, using hormone concentrations and glucose turnover as indicators of hypoglycemia counterregulation. Compared with control, N− studies resulted in significantly blunted epinephrine and norepinephrine responses to subsequent hypoglycemia. Conversely, the N+ group exhibited unimpaired hypoglycemia counterregulation, characterized by appropriate increases in epinephrine, norepinephrine, and endogenous glucose production. Thus, OR blockade with naloxone during antecedent exercise prevents the development of acute EAAF by improving the catecholamine responses and by restoring endogenous glucose production. *Diabetes* **61:1609–1615, 2012**

Maintenance of near-normal glycemia represents a major goal in the clinical management of type 1 diabetes (1) because the role of glucose control has been well established in reducing the complications associated with diabetes (2). However, iatrogenic hypoglycemia, resulting from imperfect insulin use, is a major barrier to achieving glycemic control in people with type 1 diabetes (3). Glucagon and sympathoadrenomedullary responses serve to counterregulate hypoglycemia under normal circumstances (4), but defects in hormonal counterregulation in patients with type 1 diabetes(5) predispose them to frequent hypoglycemic events in the setting of exogenous insulin therapy (3,4). Repeated episodes of hypoglycemia lead to further deterioration in the hypoglycemic counterregulatory response, which results in a syndrome termed hypoglycemia-associated autonomic failure (HAAF) (5,6). HAAF is manifested biochemically by blunted glucagon and sympathoadrenomedullary hormonal responses to hypoglycemia and clinically presents as hypoglycemia unawareness and impaired recovery from hypoglycemic events (6).

The regulation of glucose during and after exercise in people with type 1 diabetes is subject to similar counterregulatory mechanisms and inadvertent insulin excess (7). As a result, hypoglycemia frequently occurs during and after exercise in these patients (8,9), which is a major impediment to physical activity in this group. Experiments using antecedent exercise have shown attenuation of the counterregulatory response during subsequent hypoglycemia in normal and type 1 diabetic subjects (10,11), thereby resulting in exercise-associated autonomic failure (EAAF). The similarities between HAAF and EAAF suggest that they may share a common pathophysiology.

The mechanisms underlying HAAF and EAAF have not been yet definitively worked out. Thus far, cortisol, brain glycogen stores, and endogenous opioids have been implicated, at least in part (6,12,13). We have demonstrated that blockade of endogenous opioid receptors with naloxone during antecedent hypoglycemia in nondiabetic adults prevents development of HAAF during subsequent hypoglycemia (12), suggesting a mechanistic role for endogenous opioids. Because hypoglycemia and exercise both induce the release of endogenous opioids (14,15), including β-endorphin, we hypothesized that opioid receptor blockade during antecedent exercise would also prevent EAAF.

RESEARCH DESIGN AND METHODS

We studied 8 healthy volunteers (5 men, 3 women, age 28 ± 5.3 years, BMI 25.2 ± 5.7 kg/m², HbA$_{1c}$ 5.4 ± 0.5%). The inclusion criteria required that the subjects had no history of hypoglycemia and did not exercise for 2 weeks before the study. Each subject participated in three different sets of studies, in random order, separated by at least 5 weeks between studies. All studies were performed after an overnight fast. Each set of studies consisted of 2 consecutive days. Day 1 in each set consisted of two 90-min hyperinsulinemic-euglycemic clamps, with plasma glucose maintained at 5 mmol/L. During the clamp phases, each subject was assigned to one of the following study conditions: *1*) 90 min of exercise on a stationary ergonomic bicycle performed at 60% of $V_{O_{2max}}$, with naloxone infusion (N+); *2*) 90 min of exercise on a stationary ergonomic bicycle performed at 60% of $V_{O_{2max}}$, with normal saline infusion replacing the naloxone (N−); or *3*) 90 min of rest with naloxone infusion (control). The insulin clamp and the glucose infusion were necessary to maintain similar plasma glucose and insulin concentrations in all studies, enabling us to selectively assess the effects of the naloxone infusion during antecedent exercise on subsequent hypoglycemia counterregulation. Day 2 was identical in all studies and included a hyperinsulinemic stepped hypoglycemic clamp, with quantification of hormonal responses and glucose kinetics.

At least 2 weeks before the initial study, all subjects were admitted to the Clinical Research Center to determine their $V_{O_{2\ max}}$. Incremental exercise was performed on a stationary cycle ergometer, and expired gases were collected and analyzed using computerized open-circuit indirect calorimetry (Sensor-Medics VMax-29, Yorba Linda, CA), as previously described (16,17). $V_{O_{2\ max}}$ averaged 37 ± 4.2 mL/kg/min.

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. At 0800 h on the study day, two indwelling cannulae were inserted in all subjects. 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. At t −30 min, a constant insulin infusion (Humulin Regular; Eli Lilly, Indianapolis, IN) was initiated at a rate of 1 μU/kg/min, and a variable infusion of 20% dextrose was administered to maintain the plasma glucose concentration at euglycemia throughout the study. Blood samples were collected at 5-min intervals for measurements of plasma glucose. At $t = 0$ subjects were assigned to 90 min of 1) exercise with naloxone (N+) (Narcan; Du Pont Pharmaceuticals, Wilmington, DE), administered as a primed continuous infusion at 0.4 μg/kg/min; 2) exercise without naloxone (N−); or 3) rest with naloxone (control), administered as a primed continuous infusion at 0.4 μg/kg/min, depending on which protocol the subject was randomized to on the study day.

At the completion of the 90-min clamp, the insulin and naloxone infusions were discontinued, and the plasma glucose was maintained at euglycemia with the infusion of dextrose, as needed, for 90 min. During this time, the subjects also received a snack containing 15 g of carbohydrate. At $t = 180$ min, the experimental conditions were resumed, with subjects assigned to the same conditions as during the first 90 min. At the completion of the second clamp, a meal was provided, glucose was stabilized, intravenous cannulae were removed, and the subjects were discharged. In all the groups, blood samples were obtained for the determinations of serum β-endorphin.

Day 2. At 0800 h, the subjects had two indwelling cannulae inserted. At $t = -120$ min, a primed-continuous infusion of high-performance liquid chromatography–purified [3-³H] glucose was initiated with a bolus of 21.6 μCi, followed by a continuous infusion of 0.15 μCi/min for the entire study period. The specific activity of infused dextrose was kept equivalent to plasma glucose–specific activity by the addition of [3-³H] glucose to the infusate, as previously described by Finegood et al. (18). 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 0.5 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. At $t = 50$ min, and every 50 min thereafter, the plasma glucose concentration was decreased by decrements of 10 mg/dL for 50 min each 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 5.0, 4.4, 3.9, and 3.3 mmol/L. Blood samples were obtained for the determinations of plasma insulin, C-peptide, glucagon, epinephrine, norepinephrine, and cortisol, as well as for glucose turnover.

Plasma glucose was measured with a Beckman glucose analyzer (Beckman Coulter, Fullerton, CA), using the glucose oxidase method. Plasma [3-³H] glucose radioactivity was measured in duplicate on the supernatants of barium hydroxide–zinc sulfate precipitates of plasma samples, after evaporation to dryness to eliminate tritiated water (19). The methods for measurement of plasma insulin, C-peptide, glucagon, cortisol, and their intra- and interassay variations have been previously reported (20). Plasma β-endorphin was
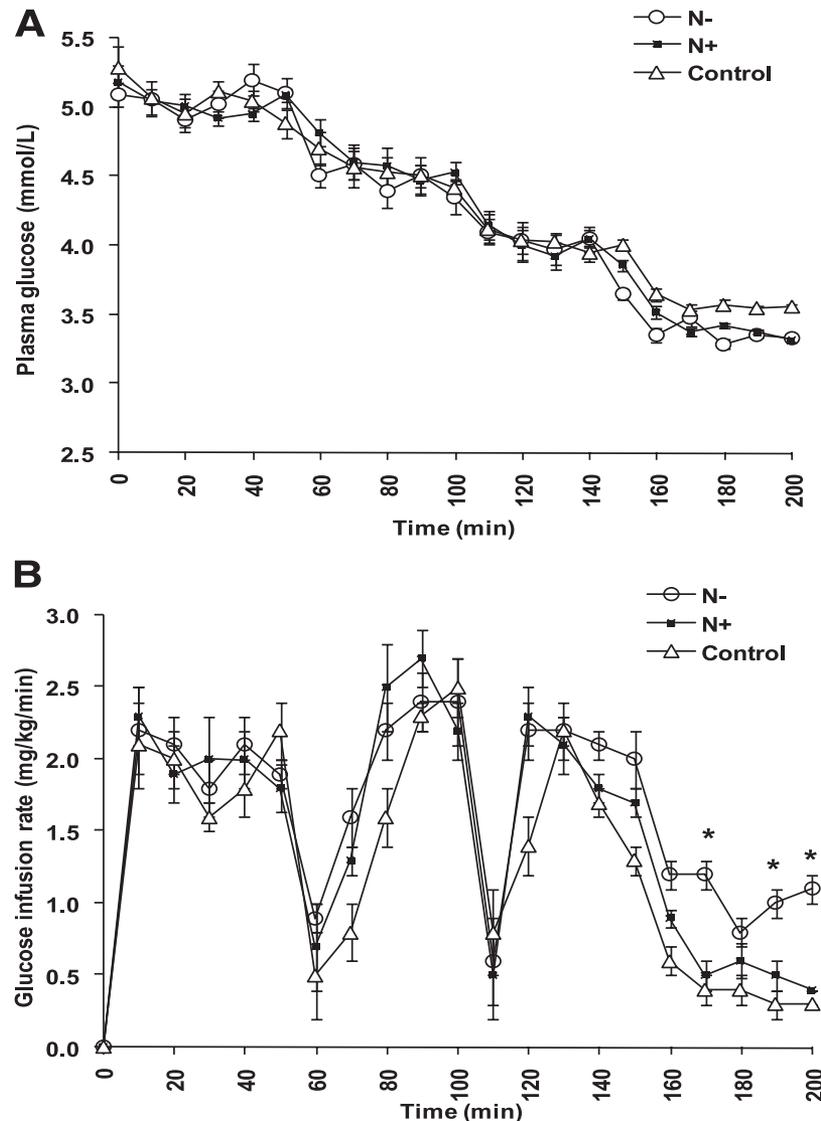
FIG. 1. Plasma glucose concentrations (day 2) at each glucose step (*A*) and glucose infusion rates (day 2) at each glucose step (*B*) in the N−, N+, and control studies. *P < 0.01 for N− vs. N+ and control.

measured using an enzyme-linked immunosorbent assay (MD Bioproducts, St. Paul, MN). Plasma epinephrine and norepinephrine levels were determined using radioimmunoassay (IBL-America, Minneapolis, MN).

Statistical analysis. The data are presented as the mean ± SEM. Steele's equation was used for calculation of glucose turnover, as described (21). Values for endogenous glucose production (EGP) and glucose uptake, obtained at 10-min intervals, were averaged over the final 30 min of each glucose step. Statistical analyses were performed using repeated-measures ANOVA for multiple comparisons and the paired Student t test for comparisons between two means (same subject) before and after an intervention (naloxone infusion). A value of $P < 0.05$ was considered significant.

RESULTS

Day 1. Plasma β-endorphin levels at baseline ($t = 0$) were $5.7 ± 1.3$, $7.2 ± 1.8$, and $5.2 ± 0.9$ pg/mL in the N−, N+ and control studies, respectively. At the end of the studies ($t = 270$ min), plasma β-endorphin concentrations increased significantly in the N− and N+ studies ($41.7 ± 5.1$ and $36.2 ± 4.6$ pg/mL, respectively; $P < 0.001$ compared with baseline for both sets of studies), but remained unchanged in the control studies ($9.7 ± 1.2$ pg/mL, $P =$ NS compared with baseline).

Day 2. Plasma glucose concentrations during the hyperinsulinemic stepped hypoglycemic clamps on day 2 are shown in Fig. 1A. All study protocols (N−, N+, and control)

achieved the target plasma glucose levels, with no significant differences among the studies.

Glucose infusion rates are depicted in Fig. 1B. During the first (5.0 mmol/L), second (4.4 mmol/L), and third (3.9 mmol/L) target glucose steps, average glucose infusion rates (mg/kg/min) were comparable in the N− ($2.1 ± 0.2$), N+ ($2.1 ± 0.2$), and control ($1.9 ± 0.1$) studies ($P =$ NS). However, during the 3.3-mmol/L glucose step, the mean rate of glucose infusion (in mg/kg/min) was $1.0 ± 0.1$ in the N−, $0.5 ± 0.1$ in N+, and $0.3 ± 0.1$ in control studies ($P < 0.01$ for N− vs. the N+ and control studies).

Plasma insulin concentrations were similar in all studies at baseline, averaging (in pmol/L) $42.4 ± 4.2$ in the N−, $57.64 ± 3.5$ in the N+, and $47.9 ± 3.5$ in the control studies ($P =$ NS). Similarly, there was no significant difference in plasma insulin concentration during all clamps, averaging $357.7 ± 25$ in the N−, $366 ± 23.6$ in the N+, and $367.4 ± 29.9$ in the control studies ($P =$ NS; Fig. 2A). Plasma C-peptide concentrations were comparable in all sets of studies at baseline (averaging $0.46 ± 0.1$ nmol/L) and during the hypoglycemic nadir (averaging $0.04 ± 0.01$ nmol/L; Fig. 2B).

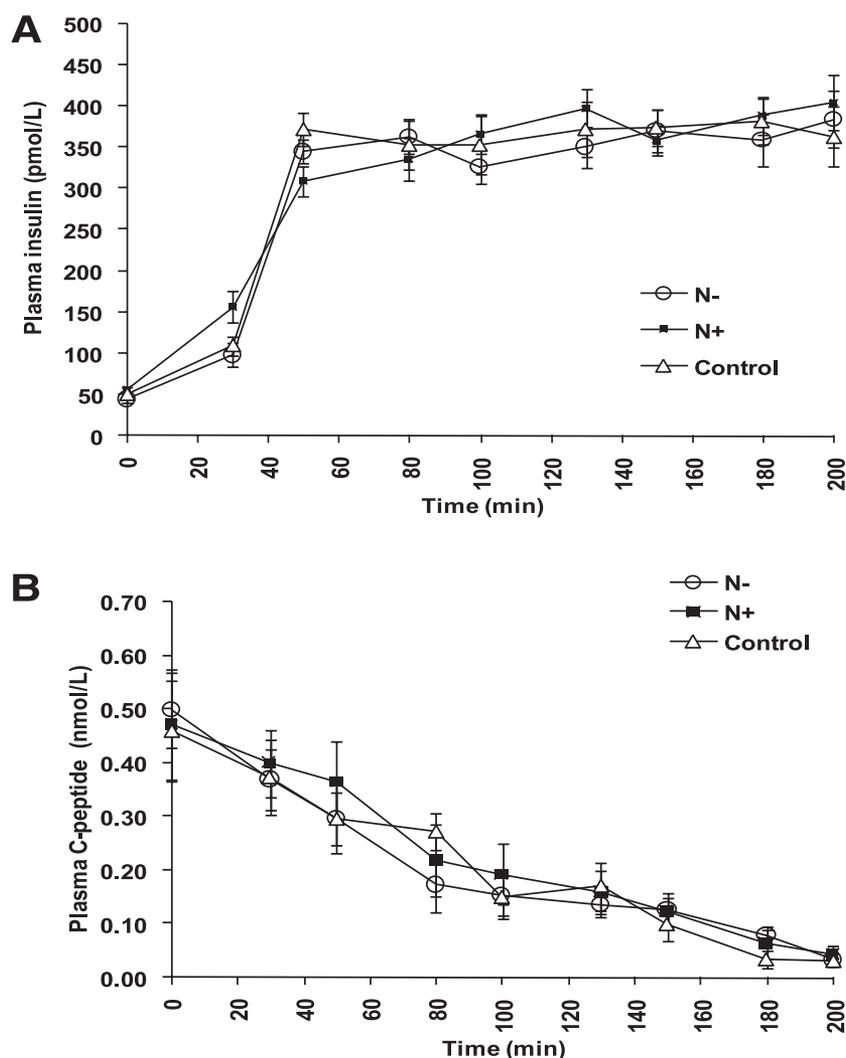Plasma epinephrine concentrations were similar in all studies during the 5.0 and 4.4 mmol/L glucose steps

FIG. 2. Concentrations of plasma insulin (A) and plasma C-peptide (B) are shown on day 2 in the N−, N+, and control studies.

(306.3 ± 60.6, 365.2 ± 46.4, and 284.4 ± 46.4 pmol/L in the N−, N+, and control studies, respectively; P = NS). Further reduction in plasma glucose to 3.3 mmol/L was associated with increments in plasma epinephrine in all studies; however, a significantly lower plasma epinephrine concentration (in pmol/L) was demonstrated in the N− studies (2,538.4 ± 256.6) compared with the N+ (3,832.2 ± 480.4) and control studies (4,192.5 ± 502.2; $P < 0.01$, Fig. 3A).

Plasma norepinephrine concentrations were equivalent during the 5.0 and 4.4 mmol/L glucose steps in all studies (Fig. 3B). However, during the 3.3 mmol/L glucose step, plasma norepinephrine (in pmol/L) increased only slightly in the N− studies (1,672.8 ± 443.3 pmol/L) compared with the N+ (3,322 ± 461.1) and control studies (2,873 ± 366, $P < 0.05$ for N− vs. N+ and control).
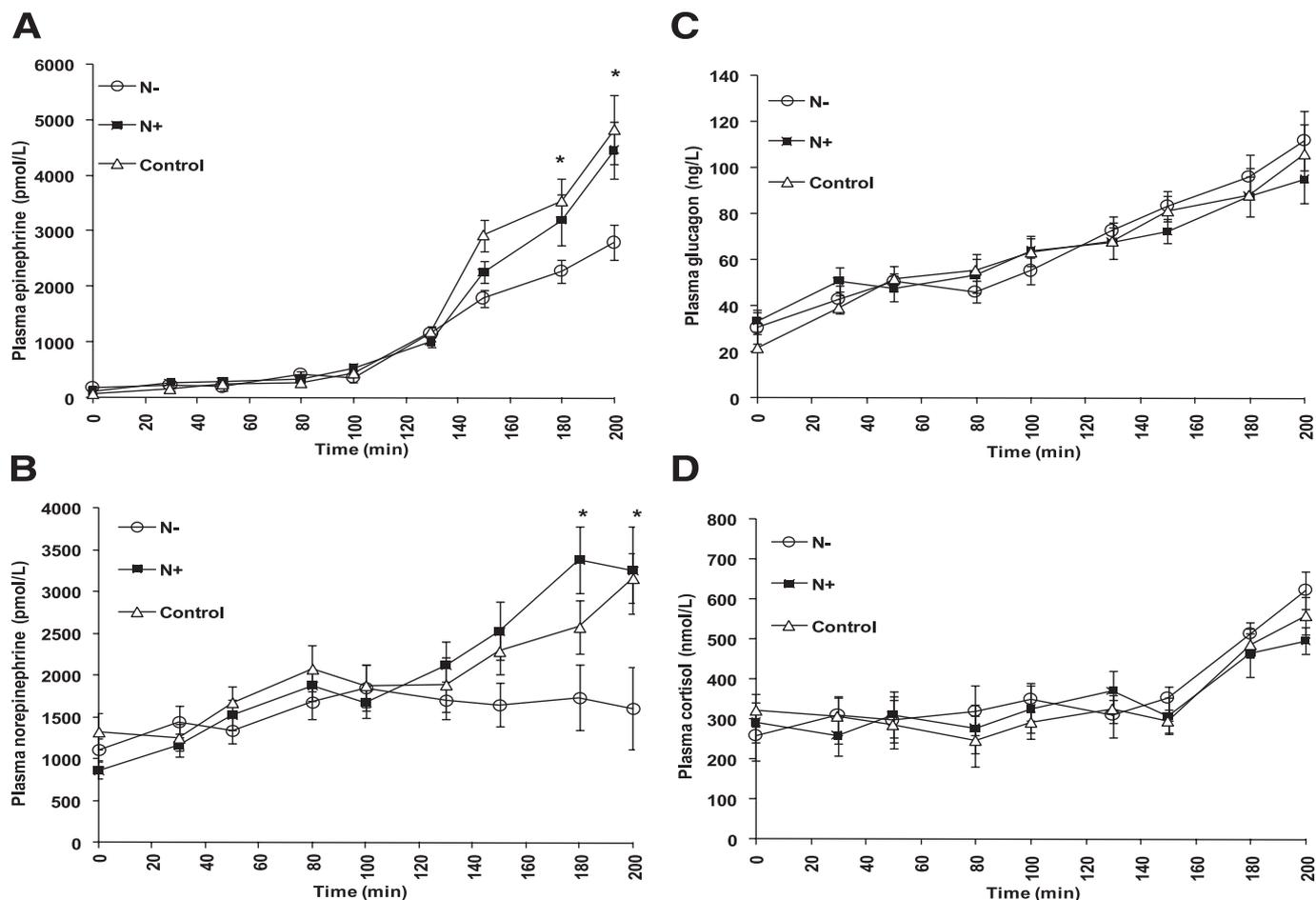
Plasma glucagon and cortisol concentrations were equivalent in all studies at baseline and increased similarly with hypoglycemia in the N−, N+ and control studies (Fig. 3C and 3D).

Mean baseline EGP was similar in all studies (2.2 ± 0.2, 2.1 ± 0.2, and 2.1 ± 0.2 mg/kg/min, in the N−, N+, and control studies, respectively; P = NS). With the initiation of insulin infusion, EGP was equally suppressed by ~65% in all studies. During the 3.3 mmol/L glucose step, EGP recovered by 53% in the N− studies and by 92% and 85% in the N+ and control studies, respectively ($P < 0.01$ vs. N−; Fig. 4).

DISCUSSION

We provide data that antecedent vigorous exercise can induce EAAF during subsequent hypoglycemia in healthy adults and that concomitant blockade of opioid receptors with naloxone during exercise prevents the development of EAAF by averting the decrements in epinephrine and norepinephrine responses and restoring the EGP to nearly normal levels. The normalization of EGP during the hypoglycemic phase was also associated with a lower glucose infusion rate required to maintain plasma glucose levels at goal, further supporting our findings of improved glucose counterregulation and recovery from hypoglycemia when opioid receptor blockade coincided with antecedent exercise. Thus, our findings suggest that endogenous opioids, including β-endorphin, may play a significant role in the pathogenesis of EAAF.

Several studies in healthy subjects also have shown blunting of the hypoglycemic counterregulatory response after antecedent exercise (22,23). In addition, experimental evidence suggests that opioid receptor blockade with naloxone administration during exercise results in the rise of epinephrine and norepinephrine in nondiabetic individuals (24,25). However, to the best of our knowledge, we are the first to demonstrate that naloxone administration during antecedent vigorous exercise prevents the attenuation of the catecholamine response during subsequent hypoglycemia, thus abrogating the development of EAAF.

FIG. 3. Concentrations of plasma epinephrine (A), norepinephrine (B), glucagon (C), and cortisol (D) are shown on day 2 at each glucose step in the N−, N+, and control studies. *$P < 0.05$ for N− vs. N+ and control.
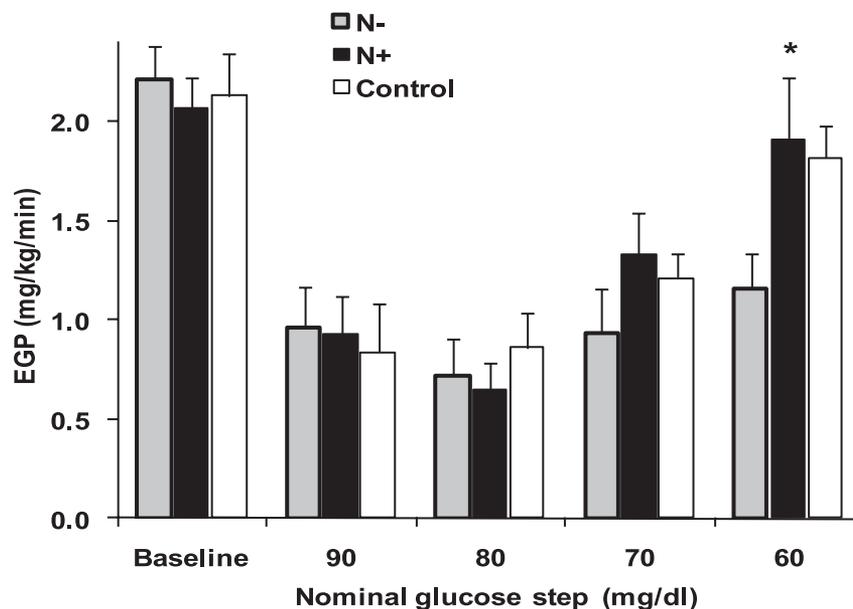
FIG. 4. EGP on day 2 averaged for the final 30 min of each glucose step in the N−, N+, and control studies. *P < 0.01 for N− vs. N+ and control.

Because catecholamine blunting is also known to occur during hypoglycemia after exercise in type 1 diabetic subjects (10,11), opioid receptor blockade may have a potential role in the prevention of EAAF in this group.

That we did not observe a decrement in glucagon levels during hypoglycemia that followed the exercise period is not necessarily surprising given the findings of other investigators. Hypoglycemia-induced secretion of glucagon was the same in antecedent exercise groups compared with control subjects in a number of studies of healthy subjects (23,26). However, a decline in glucagon levels during hypoglycemia was observed in nondiabetic people with repeatedly induced hypoglycemia (12). Although the explanation for these discrepant findings is not obvious, there are physiologic differences between hypoglycemia and exercise that may provide some clues. Exercise does not reliably induce a rise in peripheral circulating glucagon levels (in contrast to hepatic portal levels), which is typically always observed during acute hypoglycemia in healthy people (27); thus, the lack of effect on glucagon during antecedent exercise may have an influence on its levels during subsequent hypoglycemia. Hence, a different mechanism is likely to be involved in the attenuation of the glucagon response, as opposed to the catecholamine response, as they relate to HAAF and EAAF.

Evidence is accumulating for the role of endogenous opioids, including β-endorphin, in exercise and the pathogenesis of EAAF. The level of β-endorphin rises in response to exercise (14,28), as it is presumably released from the proopiomelanocortin neurons of the pituitary (29) and the adrenal medulla (30,31). The stimuli for β-endorphin release during exercise may be anaerobic metabolism (14), exercise intensity and duration (14), or catecholamine stimulation of the adrenergic receptors (31). Finally, β-endorphin has been implicated in the analgesic response and hormonal regulation of glucose metabolism under normal exercise conditions (14).

β-Endorphin may manifest its effect on glucose regulation during subsequent hypoglycemia and EAAF via actions on the opioid receptors in the central nervous system (CNS) and periphery. In the CNS, opioids bind to δ-, κ-, and μ-receptors in areas of the thalamus and hypothalamus responsible for glucose sensing, including the ventromedial hypothalamus, arcuate nucleus, and dorsal medial thalamus (32–37). Administration of exogenous β-endorphin into the rat brain was associated with the suppression of hypothalamic responses to hypoglycemia (38). In addition, repeated induction of hypoglycemia in rats, which is associated with β-endorphin release (12,15), resulted in the suppression of transcription of hypothalamic genes that regulate the transition from glycolysis to fatty acid oxidation (39). This transition may be an important mechanism used to maintain adequate glucose levels during hypoglycemia and contribute to recovery from hypoglycemia. The effect on the suppression of gene transcription is reversed with naloxone administration during antecedent hypoglycemia (39), thereby implying that endogenous opioids are the mediators of this effect.

Peripherally, β-endorphin may exert its influence on hypoglycemia counterregulation via actions at the adrenal medulla. Animal data suggest that β-endorphin released by the adrenals may induce glucose utilization via upregulation of the GLUT 4 gene expression and suppress hepatic gluconeogenesis via downregulation of PEPCK gene expression (40,41). Peripheral administration of exogenous β-endorphin induced a similar response in rats (42). If analogous events occur in humans, they would be expected to prevent recovery from hypoglycemia and result in HAAF and EAAF.

The release of adrenal β-endorphin during exercise is likely mediated via α_1-adrenoreceptor stimulation by catecholamines, as suggested by phenylephrine-induced secretion of β-endorphin and a decline in β-endorphin levels after the administration of α-adrenergic antagonists in rats (31,41). Intra-adrenal opioid secretion subsequently suppresses catecholamine release from the adrenals (43,44) by stabilizing the actin filaments of the chromaffin cells (43), thus implying the existence of a negative-feedback mechanism between adrenal β-endorphin and catecholamine release. This negative-feedback mechanism may generally serve to protect the organism from deleterious cardiovascular effects associated with chronic exposure to elevated catecholamine levels, as may occur with repetitive

stresses or exercise. The suppression of the adrenal stress response may serve a beneficial role under chronic stress conditions but may also be responsible for the disabling symptoms of EAAF in patients with type 1 diabetes.

Naloxone is an opioid receptor antagonist characterized by high affinity to the μ-opioid peptide receptors in the brain. In vitro, naloxone reverses opioid effects on the adrenal glands (44), thereby suggesting that peripheral opioids may have a pathogenic role in HAAF and EAAF. These findings are further supported by human studies, in which pretreatment with an adrenergic antagonist during antecedent hypoglycemia prevented the attenuation of the catecholamine response during subsequent hypoglycemia (45). The mechanism responsible for preserving the hypoglycemia-induced catecholamine release in the setting of adrenergic blockade during antecedent hypoglycemia likely results from inhibition of α_1-adrenergic stimulation of intra-adrenal β-endorphin release, as evidenced by the presence of an opioid antagonist or the μ-receptor knockout preventing the glucose-lowering effect of adrenergic stimulation (40,41). Although most of this evidence is obtained in models of HAAF, given the significant pathophysiologic similarities between HAAF and EAAF and the shared induction of β-endorphin release in response to hypoglycemia and exercise, we speculate that these mechanisms may be applicable to EAAF as well.

In conclusion, we have shown that opioid receptor blockade with naloxone during antecedent exercise can prevent the onset of EAAF during subsequent hypoglycemia in healthy subjects. Although the response to opioid blockade during exercise cannot be predicted in patients with type 1 diabetes, because this group has been reported to have a decreased exercise-induced release of β-endorphin (46), the effect of opioid receptor blockade on EAAF ought to be evaluated in type 1 diabetes because EAAF remains a significant risk for this population.

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