Prolactin and β-Endorphin Responses to Hypoglycemia Are Reduced in Well-Controlled Insulin-Dependent Diabetes Mellitus

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Several pituitary hormones, including corticotropin (ACTH), growth hormone (GH), prolactin, and β-endorphin (but not thyrotropin, follicle-stimulating hormone, or luteinizing hormone), are released in response to hypoglycemia in normal subjects. In patients with insulin-dependent diabetes mellitus (IDDM), the degree of glycemic control is known to alter ACTH and GH responses to hypoglycemia. The current study was performed to examine the effect of glycemic control on prolactin and β-endorphin responses to hypoglycemia in subjects with IDDM. We performed 3-hour stepped hypoglycemic-hyperinsulinemic clamp studies (12 pmol/kg/min) during which plasma glucose was decreased from 5.0 mmol/L to 2.2 mmol/L in steps of 0.6 mmol/L every 30 minutes in 20 subjects with uncomplicated IDDM (12 males and eight females; age, 26 ± 2 years; IDDM duration, 10 ± 1 years; body mass index, 23.6 ± 0.6 kg/m^2) and 10 healthy subjects (five males and five females aged 30 ± 1 years). The 10 diabetic subjects in good glycemic control (mean hemoglobin A_1 [HbA_1], 7.5% ± 0.3%; normal range, 5.4% to 7.4%) were compared with the 10 poorly controlled patients (mean HbA_1, 12.6% ± 0.5%; $P < .001$ *v* well-controlled diabetic group). During hypoglycemia, prolactin levels in the well-controlled diabetic group did not change (7 ± 1 μg/L at plasma glucose 5.0 mmol/L to 9 ± 2 μg/L at plasma glucose 2.2 mmol/L), whereas prolactin levels increased markedly in the poorly controlled diabetic group (7 ± 2 μg/L to 44 ± 17 μg/L) and healthy volunteers (12 ± 2 μg/L to 60 ± 19 μg/L, $P < .05$ between IDDM groups). The plasma glucose threshold required for stimulation of prolactin secretion was 2.2 ± 0.1 mmol/L in well-controlled IDDM, 3.0 ± 0.4 mmol/L in poorly controlled IDDM, and 2.4 ± 0.1 mmol/L in healthy subjects ($P < .05$ between IDDM groups). Responses in males and females were similar. The increase in β-endorphin levels was also attenuated in well-controlled IDDM patients (4 ± 1 pmol/L at plasma glucose 5.0 mmol/L to 11 ± 4 pmol/L at plasma glucose 2.2 mmol/L) versus poorly controlled IDDM patients (5 ± 1 pmol/L to 26 ± 7 pmol/L) and healthy subjects (8 ± 1 pmol/L to 56 ± 13 pmol/L). The plasma glucose threshold required for stimulation of β-endorphin release was again lower in well-controlled IDDM versus poorly controlled IDDM patients (2.2 ± 0.1 *v* 3.0 ± 0.3 mmol/L) and healthy subjects (2.5 ± 0.4 mmol/L, $P < .05$ between IDDM groups). In conclusion, prolactin and β-endorphin responses to a standardized hypoglycemic stimulus (plasma glucose, 2.2 mmol/L) are reduced and plasma glucose levels required to stimulate release of prolactin and β-endorphin are lower in well-controlled IDDM compared with poorly controlled IDDM and healthy subjects. Thus, stress hormones not previously considered to have a primary role in plasma glucose recovery from hypoglycemia are affected by glycemic control, suggesting a more generalized alteration of hypothalamic-pituitary responses to hypoglycemia in IDDM patients with strict glycemic control.

STRICT GLYCEMIC CONTROL of insulin-dependent diabetes mellitus (IDDM) is associated with defective counterregulatory hormone responses to hypoglycemia. Many studies have shown that epinephrine and cortisol responses to hypoglycemia are reduced and/or occur at a lower glucose level in subjects with well-controlled IDDM.[1-6] Some studies also have shown a reduction in growth hormone (GH) and corticotropin (ACTH) responses to hypoglycemia in such subjects.[7-9] Recurrent exposure to hypoglycemia is the likely mechanism by which these adaptations occur, since defective counterregulatory hormonal responses and altered glucose thresholds similar to those seen in IDDM subjects in strict glycemic control can be induced in normal and IDDM subjects by exposing them to as few as two episodes of hypoglycemia.[10-14]

The exact mechanism by which these adaptations occur in humans remains uncertain. Data from animals suggest that exposure to recurrent hypoglycemia induces adaptations at the blood-brain barrier that maintain glucose transport to the brain despite peripheral hypoglycemia, and thus lessen cerebral neuroglycopenia.[15,16] Recent studies suggest that a similar mechanism may occur in humans.[17,18] These adaptations in counterregulatory hormone responses appear to be largely specific for hypoglycemia alone, since most hormonal responses to nonhypoglycemic stimuli remain intact.[19-21]

The full extent of the modification in the response of the hypothalamic-pituitary axis to hypoglycemia in IDDM remains uncertain. Autonomic nervous stimulation of adrenal medullary epinephrine release appears to be reduced in well-controlled IDDM subjects.[22-25] The effect of strict glycemic control of IDDM on GH responses to hypoglycemia appears variable, with some studies showing a reduction in response and others showing either no effect or an increased response.[9,22] Whereas thyrotropin, follicle-stimulating hormone, and luteinizing hormone levels do not increase in response to hypoglycemia in normal subjects,[26,27] both prolactin and β-endorphin are produced in response to insulin-induced hypoglycemia.[28,29] We undertook this study to determine the effect of glycemic control in

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IDDM on prolactin and β-endorphin responses to hypoglycemia.

SUBJECTS AND METHODS

Patients and Controls

Twenty patients with IDDM and 10 healthy volunteers were studied. Ten of the diabetic subjects (six males and four females) were selected because they had well-controlled diabetes, with a hemoglobin A_1 (HbA_1) level of 9% or less (nondiabetic range, 5.4% to 7.4%). A second group of 10 diabetic subjects (six males and four females) had poorly controlled diabetes, with a HbA_1 of 11% or greater. Clinical characteristics of the subjects are shown in Table 1. Some data from the insulin clamp studies have been reported previously in other related studies by our group.[8,9,20]

None of the subjects had clinical evidence of autonomic or peripheral neuropathy based on history or physical examination. Autonomic function was further assessed by measuring the heart rate variation with slow deep breathing at a rate of five breaths per minute and in response to the Valsalva maneuver. The systolic blood pressure response to standing and the ratio of the interval between successive R waves on the electrocardiogram (RR interval) 15 and 30 seconds after standing were also assessed.[30] No patient had dipstick-positive proteinuria or other clinical or laboratory evidence of nephropathy, and none were taking medications other than insulin.

Ten healthy volunteers (five males and five females), none of whom were taking medications or had a family history of diabetes, served as controls. The three study groups did not differ statistically with regard to age or body mass index (Table 1). Voluntary informed written consent was obtained from each person before the study, and the study protocol was approved by the Joslin Diabetes Center Committee on Human Studies.

Procedures

All studies began at 8 AM after a 10- to 12-hour overnight fast. Diabetic subjects received their usual dose of insulin on the day before the study. To avoid nocturnal hypoglycemia, patients were instructed to eat a small snack if their bedtime glucose level was less than 5.6 mmol/L. Studies in diabetic patients were postponed for at least 1 week if hypoglycemia (defined as the presence of symptoms or a measured glucose level of <3.3 mmol/L) occurred during the previous 24 hours.

On the morning of the hypoglycemic insulin clamp study, a catheter was inserted into an antecubital vein for administration of test substances, and a second catheter was placed in a retrograde manner into a vein on the dorsal side of the ipsilateral hand or wrist for blood sampling. The hand was placed in a heated box (70°C) to ensure arterialization of venous blood.[31] In diabetic patients, plasma glucose was stabilized between 5.0 and 8.9 mmol/L with a low-dose insulin infusion of 0.6 to 1.8 pmol/kg · min. After collecting three baseline blood samples, we administered a primed continuous infusion (12 pmol/kg · min) of crystalline insulin (Eli Lilly & Co, Indianapolis, IN) for 3 hours. Plasma glucose levels were measured at 5-minute intervals, and the glucose clamp technique[8-10,32] was used to produce a stepwise decline in plasma glucose. Target glucose levels were 5.0, 4.4, 3.9, 3.3, 2.8, and 2.2 mmol/L at 30-minute intervals. During the final 10 minutes of each 30-minute interval, plasma samples were obtained for measurement of insulin, prolactin, and β-endorphin levels.

Table 1. Demographic Characteristics of the Study Subjects

Characteristic	Healthy Controls	IDDM Patients	
		Well-Controlled	Poorly Controlled
No.	10	10	10
Age (yr)	30 ± 6	25 ± 9	27 ± 7
Sex (male/female)	5/5	6/4	6/4
Body mass index (kg/m²)	22.4 ± 2.7	23.1 ± 1.3	24.2 ± 3.8
HbA_1 (5.4%-7.4%)	6.1 ± 0.4	7.5 ± 0.9	12.6 ± 1.6*
IDDM duration (yr)	—	11 ± 6	8 ± 5

NOTE. Data are expressed as the mean ± SD.
*$P < .001$ *v* other 2 groups.

Analyses

Plasma glucose level was measured at the bedside by the glucose oxidase method using a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin level was measured by a double-antibody radioimmunoassay.[33] In diabetic patients, free insulin was assayed by treating the plasma with polyethylene glycol to precipitate antibody-bound insulin. Total HbA_1 was determined by agar gel electrophoresis with the GLYTRAC glycosylated hemoglobin kit (Corning Medical, Palo Alto, CA) after removal of the labile component. Prolactin was assayed using a third-generation monoclonal immunoassay system (Nichols Institute, San Juan Capistrano, CA) with an intraassay coefficient of variation of 3% to 4%. β-Endorphin was determined using a two-site immunoradiometric assay (Nichols Institute) with an intraassay coefficient of variation of 4% to 5%.

The results are expressed as the mean ± SEM except for the demographic data in Table 1, which are expressed as the mean ± SD. The glucose threshold was determined as the glucose level at which each hormone achieved a sustained increment greater than 2 SD above the mean basal level for each subject. Data that were normally distributed were compared using Student's *t* test or ANOVA with repeated measures, as appropriate. For data that were not normally distributed, the Mann-Whitney *U* test and Kruskall-Wallis test were used. Results were analyzed using the Systat software package (Evanston, IL).

RESULTS

Glucose and Insulin

Glucose levels were significantly higher in well- and poorly controlled IDDM groups (6.1 ± 0.4 and 6.3 ± 0.2 mmol/L) at the beginning of the hypoglycemic insulin clamp protocol compared with the healthy controls (5.1 ± 0.1 mmol/L, $P < .05$ *v* IDDM groups). However, glucose values did not differ significantly between study groups for the remainder of the study, and nadir glucose levels were 2.3 ± 0.1 and 2.4 ± 0.1 mmol/L in well- and poorly controlled diabetic groups and 2.4 ± 0.1 mmol/L in healthy controls. Mean steady-state insulin values were 977 ± 92 pmol/L in the healthy volunteers, whereas free insulin levels were 741 ± 83 and 976 ± 154 pmol/L in well- and poorly controlled diabetic groups, respectively (*P* nonsignificant among groups).

Prolactin

Glycemic control. Basal levels of prolactin were 12 ± 2 μg/L in healthy controls and 7 ± 1 μg/L in diabetic subjects ($P < .01$). During hypoglycemia at 2.2 mmol/L, prolactin increased to 60 ± 19 μg/L in healthy volunteers and to 26 ± 9 μg/L in diabetic patients ($P = .07$). When diabetic subjects were grouped according to glycemic control, basal prolactin levels were 7 ± 1 and 7 ± 2 μg/L in well- and

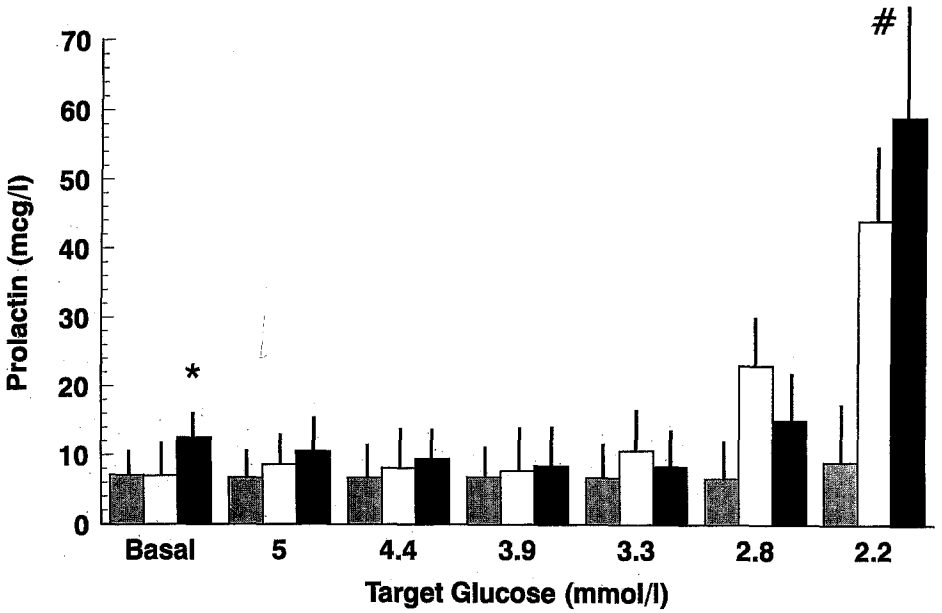

Fig 1. Prolactin levels during hypoglycemic clamp studies in 10 subjects with well-controlled IDDM (▨), 10 subjects with poorly controlled IDDM (□), and 10 healthy volunteers (■). *$P < .05$, well-controlled IDDM *v* healthy volunteers. #$P < .05$, well-controlled IDDM *v* other 2 groups.

poorly controlled patients, respectively ($P < .05$, healthy volunteers *v* each IDDM group; Fig 1). Prolactin levels during hypoglycemia (plasma glucose, 2.2 mmol/L) were 9 ± 2 and 44 ± 17 μg/L in well- and poorly controlled diabetic groups ($P < .05$, well-controlled *v* poorly controlled IDDM or healthy volunteers; Fig 1). Glucose thresholds for prolactin release were 2.4 ± 0.1 mmol/L in healthy volunteers and 2.2 ± 0.1 and 3.0 ± 0.4 mmol/L in well- and poorly controlled IDDM groups, respectively ($P < .05$ between diabetic groups). In IDDM subjects, the glucose threshold for release of prolactin was strongly correlated with glycemic control as assessed by HbA_1 ($r = .68, P < .001$; Fig 2).

Sex differences. Basal prolactin levels were 6 ± 1 μg/L in 12 male IDDM subjects and 8 ± 1 μg/L in eight female IDDM subjects (P nonsignificant between groups). Although prolactin levels at nadir glucose levels (2.2 mmol/L) were higher in female (39 ± 22 μg/L) versus male (18 ± 5 μg/L) patients, this difference did not reach statistical significance. In the healthy controls, prolactin levels at basal euglycemia (15 ± 2 *v* 10 ± 2 μg/L) and during nadir hypoglycemia (76 ± 37 *v* 43 ± 6 μg/L) were also slightly but not significantly higher in five females versus five males.

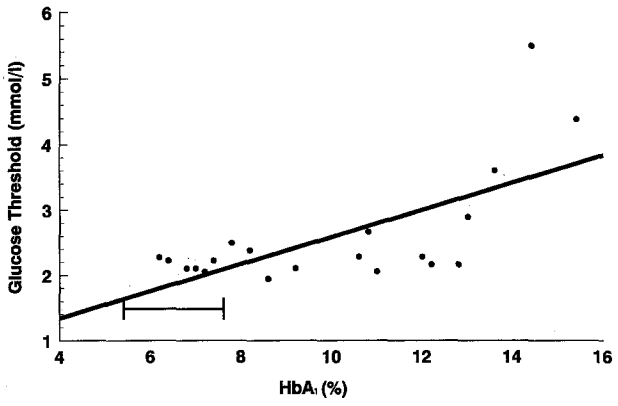

Fig 2. Relationship between the glucose threshold for release of prolactin and HbA_1 in 20 subjects with IDDM ($r = .68, P < .001$). Short horizontal line depicts normal range for HbA_1.

β-*Endorphin*

Glycemic control. Basal levels of β-endorphin were 8 ± 1 pmol/L in the healthy volunteers and 5 ± 1 pmol/L in 20 diabetic subjects ($P < .001$). β-Endorphin levels were higher in healthy controls compared with the entire diabetic group at all glucose concentrations, and this difference reached statistical significance at glucose levels of 4.4, 3.9, 2.8, and 2.2 mmol/L (all $P < .01$). At the 2.2-mmol/L glucose plateau, β-endorphin levels were 56 ± 13 pmol/L in the healthy control group and 19 ± 4 pmol/L in diabetic subjects ($P < .01$). When diabetic subjects were grouped according to glycemic control, basal levels of β-endorphin were 4 ± 1 and 5 ± 1 pmol/L in well- and poorly controlled IDDM groups, respectively ($P < .01$, healthy volunteers *v* each IDDM group; Fig 3). β-Endorphin levels were significantly higher in healthy volunteers compared with the well-controlled diabetic group at all levels of plasma glucose from 5.0 to 2.2 mmol/L ($.05 < P < .01$; Fig 3). At nadir glucose (2.2 mmol/L), β-endorphin increased to 56 ± 13 pmol/L in healthy volunteers and to 12 ± 4 and 26 ± 7 pmol/L in well- and poorly controlled diabetic groups ($P < .01$, healthy volunteers *v* well-controlled diabetic group; Fig 3). Glucose thresholds for a significant increase in β-endorphin levels were 2.5 ± 0.4 mmol/L in healthy controls and 2.2 ± 0.1 and 3.0 ± 0.3 mmol/L in well- and poorly controlled diabetic groups ($P < .05$ between IDDM groups). The glucose threshold for release of β-endorphin was strongly correlated with HbA_1 ($r = .68$, $P < .001$; Fig 4).

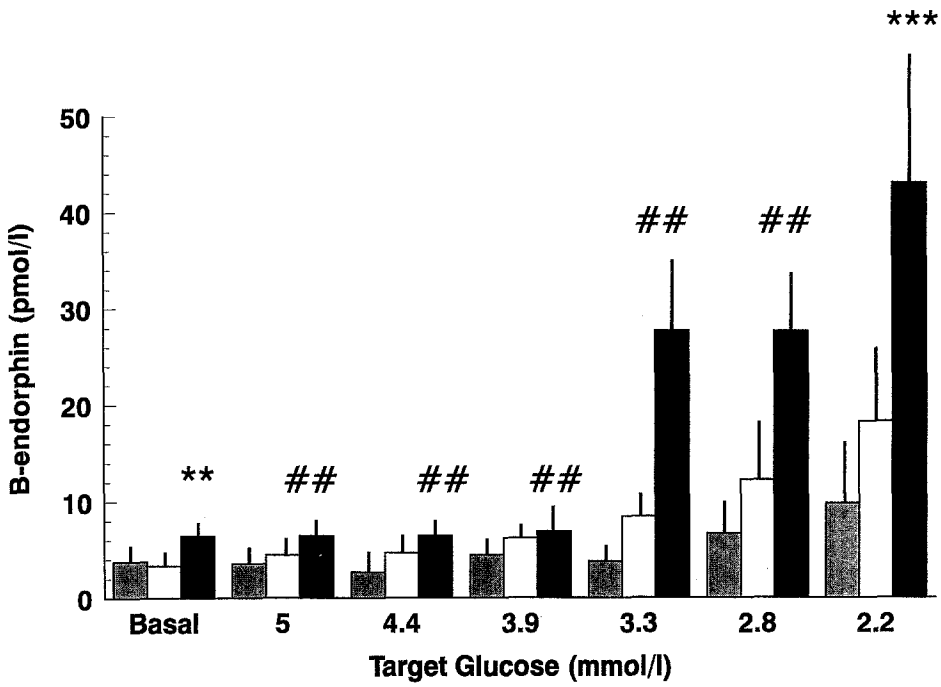

Fig 3. β-Endorphin levels during hypoglycemic clamp studies in 10 subjects with well-controlled IDDM (▨), 10 subjects with poorly controlled IDDM (□) and 10 healthy volunteers (■). **$P < .01$, healthy volunteers *v* both IDDM groups. ##$P < .05$, healthy volunteers *v* well-controlled IDDM. ***$P < .01$, healthy volunteers *v* well-controlled IDDM.

Sex differences. When diabetic subjects (12 males and eight females) were grouped according to gender, basal β-endorphin levels were 6 ± 1 pmol/L in males and 3 ± 1 pmol/L in females ($P < .05$). At nadir glucose (2.2 mmol/L), β-endorphin levels did not differ between groups (20 ± 6 pmol/L in males and 16 ± 5 pmol/L in females). In the healthy control group, basal levels of β-endorphin were 9 ± 1 and 8 ± 1 pmol/L in five males and five females. At nadir hypoglycemia of 2.2 mmol/L, levels were 78 ± 23 and 34 ± 6 pmol/L in males and females ($P = .10$).

DISCUSSION

The degree of glycemic control in patients with IDDM has profound effects on hypothalamic-pituitary-adrenal response to hypoglycemia. Well-controlled diabetic subjects usually exhibit blunted epinephrine and cortisol responses to hypoglycemia, and the glucose threshold at which these counterregulatory hormones are produced in response to hypoglycemia is also reduced.[1-6] Conversely, diabetic subjects in poor glycemic control exhibit increased catecholamine and cortisol responses and increased glucose thresholds for hypoglycemic counterregulation.[34] Exposure to recurrent hypoglycemia appears to be the factor that produces these alterations, since numerous studies have shown that exposure to as few as two episodes of hypoglycemia may induce modifications in counterregulatory hormone responses and glucose thresholds in normal subjects similar to those seen in well-controlled IDDM.[10-14] These blunted counterregulatory responses appear to be reversible by avoidance of hypoglycemia.[35-40]

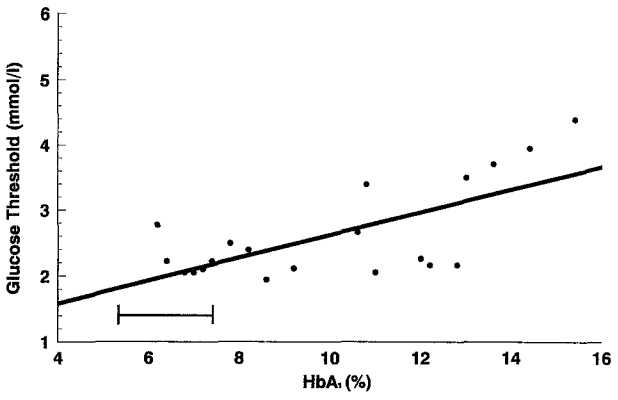

Fig 4. Relationship between the glucose threshold for release of β-endorphin and HbA_1 in 20 subjects with IDDM ($r = .68$, $P < .001$). Short horizontal line depicts normal range for HbA_1.

The full extent of the effect of recurrent hypoglycemia and strict glycemic control of IDDM on the hypothalamic-pituitary stress response to hypoglycemia remains uncertain. In normal subjects, thyrotropin, follicle-stimulating hormone, and luteinizing hormone do not increase substantially in response to insulin-induced hypoglycemia.[26,27] Conversely, prolactin and β-endorphin do increase during hypoglycemia,[28,29] although these hormones are not generally believed to play a direct role in glucose counterregulation.

Our results demonstrate a reduction in basal levels of both prolactin and β-endorphin in IDDM patients compared with healthy control subjects. Moreover, the prolactin response to hypoglycemia was lower in 10 well-controlled diabetic subjects compared with a matched group in poor glycemic control or a group of healthy volunteers. Similarly, β-endorphin levels in the well-controlled diabetic group were also lower than in the other study groups. Glucose thresholds for release of both prolactin and β-endorphin occurred at a lower glucose level in the

well-controlled IDDM group compared with the other groups. Basal levels of β-endorphin were lower in females than in males with IDDM, but prolactin and β-endorphin responses to hypoglycemia did not differ when subjects were grouped by gender. These results suggest that strict glycemic control of IDDM causes modifications in hypothalamic-pituitary stress responses to hypoglycemia beyond those directly involved in glucose counterregulation.

Plasma prolactin peaks approximately 45 minutes after an intravenous bolus injection of insulin.[41] The mechanism of prolactin release in response to hypoglycemia has not been fully elucidated, but Woolf et al[42] showed that intracellular neuroglycopenia, not increased insulin per se, is the primary stimulus. Prolactin release from pituitary lactotropes is mainly under tonic inhibitory dopaminergic control. However, the prolactin response to hypoglycemia is reduced by atropine, suggesting that autonomic inputs may be involved.[43]

Previous studies have suggested that prolactin dynamics are abnormal in subjects with IDDM. Iranmanesh et al[44] found reduced mean 24-hour prolactin levels in 11 men with poorly controlled IDDM compared with a group of healthy control subjects. Djursing et al[45] reported lower basal prolactin levels in 28 women with IDDM compared with normal healthy subjects, a finding confirmed by our data. These data may reflect increased central nervous system dopaminergic activity in IDDM subjects. There are few data on prolactin responses to hypoglycemia in IDDM subjects. Frier et al[28] found no difference in prolactin responses to hypoglycemia in 16 IDDM subjects grouped according to disease duration; the degree of glycemic control was not addressed. Our results confirm that basal prolactin levels are lower in subjects with IDDM, and suggest that strict glycemic control significantly reduces the prolactin response to hypoglycemia. The analysis also suggests that these differences cannot be explained on the basis of gender. To date, there are few data on the effect of recurrent hypoglycemia on prolactin responses in healthy subjects or IDDM patients, although a single study showed no effect of preceding mild hypoglycemia (3.3 or 3.7 mmol/L) on the prolactin response to subsequent hypoglycemia at 2.2 mmol/L in healthy subjects.[46]

β-Endorphin, an endogenous opioid, is derived with β-lipotropin and ACTH from the precursor molecule, pro-opiomelanocortin. Insulin-induced hypoglycemia causes prompt release of both β-endorphin and ACTH in normal subjects.[28,47-50] Evidence exists that β-endorphin may play a role in glucose homeostasis, but the exact nature and importance is uncertain.[51,52] Intracerebroventricular instillation of β-endorphin causes hyperglycemia, which is prevented by naloxone administration or adrenal gland denervation in rats.[53] Similarly, Ipp et al[54] showed that intracerebroventricular naloxone reduces the hyperglycemic response to central nervous system glucose deprivation, suggesting that β-endorphin augments glucose counterregulation. Suda et al[55] showed that administration of β-endorphin into the lateral ventricle in rats decreased basal corticotropin-releasing factor levels and inhibited basal and hypoglycemia-induced ACTH secretion. This inhibitory effect of β-endorphin was reversed by treatment with naloxone. Nash et al[56] showed that treatment with naloxone reduces β-endorphin and epinephrine responses to hypoglycemia in dogs. In contrast, El-Tayeb et al[57] reported that blockade of opiate receptors with intravenous infusion of naloxone increases counterregulatory hormone responses to hypoglycemia in normal dogs, suggesting an inhibitory role for β-endorphin in glucose counterregulation.

Frier et al,[28] in a study of 16 subjects with type I diabetes mellitus, reported basal levels of β-endorphin similar to those seen in our study. β-Endorphin responses to hypoglycemia in their IDDM patients were lower than those seen in the healthy control group, a finding also confirmed by our data. Wanke et al[58] studied 11 subjects with IDDM and also reported that basal β-endorphin levels were lower in the IDDM group compared with healthy controls. They also found that the β-endorphin response to a nonhypoglycemic stimulus (breathing against resistive loads) was reduced in IDDM versus healthy subjects. Based on these two studies and the data from our study, it appears that basal β-endorphin levels and the responses to hypoglycemic and nonhypoglycemic stimuli may be reduced in subjects with IDDM. Our data also suggest that β-endorphin levels are lowest in IDDM subjects in good glycemic control.

Caprio et al[59] studied counterregulatory responses to hypoglycemia following intravenous naloxone in healthy volunteers and eight well-controlled IDDM subjects. When naloxone was infused during hypoglycemia, the responses of epinephrine, cortisol, GH, and glucose production were greater than with hypoglycemia alone. It was suggested that as endogenous opiates are released during hypoglycemia, they might exert a modulatory effect on glucose counterregulation by attenuating adrenal medullary hormone responses. Our data suggest an alternate hypothesis, since β-endorphin levels in response to hypoglycemia were lowest in our well-controlled IDDM group and the glucose threshold for release of β-endorphin was equal to or less than that reported for epinephrine in well-controlled IDDM patients.[9] Thus, release of β-endorphin is not likely to affect the epinephrine response to hypoglycemia. Although β-endorphin may have a minor modulatory role in the hypothalamic-pituitary and sympathoadrenal responses to hypoglycemia, our data suggest that the degree of glycemic control is a more important direct regulator of the β-endorphin response to hypoglycemia.

Strict glycemic control of IDDM has now been shown to be associated with altered responses and glucose thresholds for epinephrine, norepinephrine, cortisol, GH, ACTH, and (in the current study) prolactin and β-endorphin. Boyle et al[17,18] demonstrated that exposure to prolonged hypoglycemia in normal volunteers or strict glycemic control in IDDM patients is associated with alterations in brain glucose uptake. Brain glucose uptake is maintained at the peripheral glucose levels at which it had been compromised before hypoglycemic exposure. This modification limits cerebral neuroglycopenia and thus reduces the neuroglycopenic stress at a given peripheral glucose level. This

adaptation results in decreased hormonal counterregulation to a given degree of hypoglycemia. Data from animal studies suggest that upregulation of GLUT-1 transporter number at the blood-brain barrier may be the mechanism by which the hypoglycemic adaptation occurs.[60,61] Our data on prolactin and β-endorphin responses suggest that the adaptation in hypothalamic-pituitary-adrenal and central autonomic responses to hypoglycemia in IDDM subjects in strict glycemic control is not limited to the hormones directly involved in glucose counterregulation. It remains to be determined whether β-endorphin plays a significant role in glucose counterregulation in IDDM.

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