Hormone-independent activation of EGP during hypoglycemia is absent in type 1 diabetes mellitus

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Mevorach, Michéle, Jonathan Kaplan, Chee Jen Chang, Luciano Rossetti, and Harry Shamoon. Hormone-independent activation of EGP during hypoglycemia is absent in type 1 diabetes mellitus. Am. J. Physiol. Endocrinol. Metab. 278: E421–E429, 2000.—It has been suggested that insulin-induced suppression of endogenous glucose production (EGP) may be counteracted independently of increased epinephrine (Epi) or glucagon during moderate hypoglycemia. We examined EGP in nondiabetic (n = 12) and type 1 diabetic (DM1, n = 8) subjects while lowering plasma glucose (PG) from clamped euglycemia (5.6 mmol/l) to values just above the threshold for Epi and glucagon secretion (3.9 mmol/l). Individualized doses of insulin were infused to achieve euglycemia above the threshold for Epi and glucagon secretion (3.9 mmol/l). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The depth of hypoglycemia activates each component at a differing glycemic threshold as well as modulating its magnitude (35, 43). Among the important components of the counterregulatory cascade during moderate hypoglycemia are the secretion of glucagon and epinephrine, both of which activate endogenous glucose production (EGP) (9, 15, 16). A large body of evidence indicates that the defective secretion of these two hormones is critical to the persistent suppression of EGP and consequently the development of more severe hypoglycemia (8). Defective glucose counterregulation in type 1 diabetes is due, in large part, to the inability of EGP to overcome insulin-induced suppression and is associated with impaired secretion of glucagon and epinephrine (9, 28). The defect in epinephrine is manifested as both delay (with respect to the hypoglycemic threshold level for) and reduction (with respect to the magnitude) of hormone secretion. Recent studies from this and other laboratories suggest that extrahepatic tissues (e.g., skeletal muscle) may partially compensate for defective hepatic counterregulation (7, 9), although the mechanism underlying this effect also appears to be largely epinephrine dependent (22).

Because hormone-dependent hypoglycemia counterregulation is clearly impaired in type 1 diabetes, we hypothesized that hormone-independent activation of EGP during impending hypoglycemia might likewise be defective. The studies supporting the existence of alternative, nonhormonal mechanisms by which EGP might be activated [e.g., by direct neural innervation (17, 38) or by so-called “autoregulation” by glucose per se] are inconclusive (8, 23, 42). Saccà et al. (42) suggested that a nonhormonal signal stimulated EGP during a fall in plasma glucose between ~3.9 and ~3.1 mmol/l in nondiabetic human subjects. In addition, they concluded that either direct neural innervation or glucose per se might be operative (42). In type 1 diabetics, however, they found impairment of EGP but only under conditions associated with defective glucagon secretion (19, 42). Thus the role of defects in hormone-independent activation of EGP in the pathogenesis of hypoglycemia in type 1 diabetes has not been specifically examined.

In nondiabetic human subjects, Bolli et al. (4), using steady-state pancreatic/pituitary clamps, suggested that glucose per se did not activate EGP except during
severe hypoglycemia (−1.9 mmol/l). However, since combined α- and β-adrenergic blockade was used in their experimental model, a role for an epinephrine-independent mechanism was not elucidated. Hansen et al. (23) also concluded that a low glucose per se did not activate EGP during mild hypoglycemia (−3.6 mmol/l), although somatostatin without glucagon replacement was used in those experiments, resulting in a glucagon-deficient metabolic milieu that might have altered glucose fluxes. Moreover, all these previous reports used isotopic techniques to evaluate glucose turnover but were not designed to maintain constant glucosespecific activity and did not use purified tracers. These methodological considerations are germane, given that nonhormonal counterregulatory factors contribute only a small portion (−20–30%) of the EGP response during hypoglycemia, and a small effect may go undetected unless experimental methods are optimized (14).

The present study was therefore designed to compare the possible role of nonhormonal activation of EGP in nondiabetic and type 1 diabetic subjects with the following experimental goals in mind: 1) to exclude the effect of hormonal counterregulation by controlling changes in glucagon and epinephrine secretion; 2) to replace insulin by use of exogenous insulin infusions to match each subject’s basal requirement, and 3) to optimize tracer methodology by use of current standards such as purified tracer and stable isotope specific activity.

MATERIALS AND METHODS

Subjects. We studied 12 healthy, nondiabetic subjects (seven men, five women) aged 28 ± 1 (SE) yr and eight C-peptide-deficient type 1 diabetic subjects (33 ± 3 yr), also equally gender distributed. The diabetic subjects had a mean duration of diabetes of 12 ± 2 yr and an HBA1c of 8.4 ± 0.4% (range 6.8–10.2%). Body mass index in the nondiabetic subjects averaged 24.7 ± 0.9 kg/m², and in diabetic subjects 26.5 ± 1.4 kg/m². The diabetic subjects did not have proliferative retinopathy, autonomic neuropathy measured with cardiovascular function tests, urinary protein excretion >2,650 µmol/24 h, or a serum creatinine >124 µmol/l. Two subjects had microalbuminuria, and three had treated hypothyroidism and were euthyroid. None was taking any chronic medications other than insulin with the exception of two subjects with microalbuminuria who took an angiotensin-converting enzyme inhibitor that was discontinued 3 days before the studies. None of the subjects had had an episode of severe hypoglycemia (9) in the previous year or frequent mild-to-moderate (9) hypoglycemic episodes in the month before the study. Informed written consent was obtained in accordance with the guidelines of the Committee on Clinical Investigations of the Albert Einstein College of Medicine.

Study design. The study was designed to examine counterregulatory mechanisms regulating EGP while controlling changes in glucagon secretion, and this assessment was made at the 3.9 mmol/l plasma glucose step. This was achieved by clamping plasma glucagon concentrations during somatostatin infusion and by clamping plasma glucose just above the threshold for activation of epinephrine release (5, 35, 43). A deeper level of hypoglycemia (3.1 mmol/l) was then induced and represented a model of glucagon-independent but epinephrine-dependent counterregulation.

All studies were performed in the postabsorptive state after an overnight fast. Nondiabetic subjects were studied on two separate occasions ≥2 mo apart, one, an "Experimental" study protocol in which sequential plasma glucose reduction (from 5.6 to 3.9 and then to 3.1 mmol/l) was achieved with modification of the glucose clamp technique (10), and the second, a euglycemic "Control" study protocol in five of the subjects, in whom plasma glucose was maintained at 5.6 mmol/l for the periods corresponding to 5.6 mmol/l and 3.9 mmol/l of the Experimental study. Type 1 diabetic subjects were studied by means of only the Experimental protocol. Twenty-four hours before the study, diabetic subjects were admitted to the General Clinical Research Center (GCRC), withdrawn from long- or intermediate-acting insulin doses, and infused with insulin (Novolin Regular, Novo-Nordisk, Princeton, NJ), prepared with albumin 1 mg/ml diluted in 0.9% NaCl) to achieve and maintain euglycemia (plasma glucose 5.6–6.7 mmol/l) without hypoglycemia (11). The overnight (2200–0700) insulin infusion rate was adjusted according to an algorithm based on hourly blood glucose measurements and averaged 1.3 ± 0.2 pmol·kg⁻¹·min⁻¹. Nondiabetic subjects were admitted to the GCRC at 0700 on the morning of the study.

On the morning of study (0730 h), after voiding, two indwelling cannulas were inserted, one in an antecubital vein for infusions and the second placed in retrograde fashion in a distal hand vein with the hand kept in a warming chamber (65°C). These cannulas were kept patent by slow infusions of 0.9% NaCl. A priming dose (22.0 µCi) of [3-3H]glucose (HPLC-purified, New England Nuclear, Boston, MA) was administered followed by a continuous infusion at 0.15 µCi/min for the remainder of the study. After 120 min, infusions of somatostatin (Bachem, King of Prussia, PA; 250 µg/h), growth hormone (Genentech, San Francisco, CA; 3.0 ng·kg⁻¹·min⁻¹), and glucagon (Eli Lilly, Indianapolis, IN, 1.0 ng·kg⁻¹·min⁻¹) were initiated and maintained throughout the study. These infusions were prepared with albumin 1 mg/ml diluted in 0.9% NaCl. In nondiabetic subjects an infusion of insulin (Novolin Regular, Novo-Nordisk, also prepared in albumin-containing saline) was begun at this time.

Plasma glucose concentrations were measured at 5-min intervals, and the rate of glucose infusion was adjusted in both groups to maintain euglycemia (5.6 mmol/l) without the need for exogenous glucose infusion. When a stable rate of insulin infusion was determined, it was kept constant for at least an additional 60 min. Thus a total period of 120 min of euglycemia with a variable insulin infusion (≤60 min), followed by a fixed insulin infusion for 60 min, preceded the experimental manipulations.

Beginning at t = 0 (Fig. 1), the previously determined, fixed, individualized "basal" rate of insulin infusion was kept constant and ambient euglycemia was maintained for another 30 min. The 60-min period of stable euglycemia is thus termed basal because no glucose was infused. At t = 30 min, the insulin infusion rate was increased by 20%, but plasma glucose was kept constant at 5.6 mmol/l with a variable infusion of a 20% dextrose solution to which tracer was added (−0.1 µCi/ml) to maintain glucose-specific activity constant (14).

During the remainder of the study, this mild hyperinsulinemia was kept constant while dextrose infusion was adjusted every 5 min to achieve and/or maintain plasma glucose targets of 5.6, 3.9, and 3.1 mmol/l sequentially, each for 90 min (identified in the text as 5.6, 3.9, and 3.1 mmol/l steps in the Experimental protocol). In the Control studies (in nondiabetic subjects only) the identical design was employed but the plasma glucose concentration was maintained at 5.6 mmol/l,
as noted above. At the completion of each study, the tracer, glucose, and hormone infusions were stopped. The insulin infusion was continued in the diabetic patients for an additional 30 min. A meal was provided and subjects were discharged. Diabetic individuals resumed their usual treatment regimens.

Blood was sampled at 15-min intervals for glucose and tritiated water-specific activities, at 30-min intervals for plasma insulin, C-peptide, glucagon, epinephrine, and norepinephrine, free fatty acids (FFA), and glycerol, and at 60-min intervals for plasma cortisol and growth hormone. Continuous indirect calorimetry was performed for 20-min periods at baseline and in the final 20 min of each glucose plateau. Protein oxidation was estimated from the urinary urea and tritiated water-specific activities, at 30-min intervals for plasma cortisol and growth hormone. Continuous indirect calorimetry was performed for 20-min periods at baseline and in the final 20 min of each glucose plateau. Protein oxidation was estimated from the urinary urea production measured from a urine sample obtained at the end of each procedure.

Methods. Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA) by use of the glucose oxidase method. Plasma [3-3H]glucose radioactivity was measured in duplicate on the supernatants of barium hydroxide-zinc sulfate precipitates (Somogyi procedure) of plasma samples, after evaporation to dryness to eliminate tritiated water. Plasma tritiated water-specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness. Plasma insulin, C-peptide, glucagon, growth hormone, and cortisol were determined by RIA (44). Plasma epinephrine and norepinephrine were measured by means of an isotope derivative assay (44, 47). Plasma FFA and glycerol were measured by means of calorimetric enzymatic methods (39, 40), and plasma lactate was measured by an enzymatic assay (18). For indirect calorimetry, airflow and O2 and CO2 concentrations in the expired and inspired air were measured by a computerized open-circuit system (Deltatrac, Sensormedics, Yorba Linda, CA). Urinary nitrogen was measured by the Kjeldahl procedure (24). Hb A1c was measured by means of ion-exchange chromatography with an upper normal limit of 6.1%.

Analyses. Rates of glucose appearance (Ra) and disappearance (Rd) were calculated by use of Steele’s steady-state equation (48). Endogenous glucose production (EGP) was determined by subtracting the rates of glucose infusion from the tracer-determined Ra. Rates of glycolysis from plasma glucose were estimated from the increment in tritiated water per unit time (dpm · ml−1 · min−1) multiplied by body water mass (ml) per [3-3H]glucose specific activity (dpm/mg), as previously validated (41). Glycogen synthetic rates were estimated as the difference between glucose disposal rate (Rd) and glycolysis from plasma glucose (41). Carbohydrate oxidation (CHO) was calculated from O2 consumption and CO2 production (corrected for protein oxidation) using the Lusk equation (31). Data for glucose turnover, carbohydrate and lipid oxidation, plasma hormones, and substrate concentrations represent the mean values during the final 60 min of the baseline euglycemic period and the final 60 min of each of the three hyperinsulinemic periods. Statistical analysis of the data over time was performed using PROC MIXED in SAS System Version 6.12 (SAS Institute, Cary, NC) (29).

random effect considered in this mixed model is the error measurement of individual subjects, and the within-individual fixed effect is the difference between groups. For averaged data, the two-sample Student’s t-test and the Wilcoxon ranked-sum tests were employed (49). All data are presented as means ± SE.

RESULTS

Plasma glucose and insulin, and glucose infusion rate. Plasma glucose and insulin during the Experimental protocol in both groups are shown in Fig. 1. Plasma glucose concentrations during the basal euglycemic period were 5.7 ± 0.2 mmol/l in nondiabetics and 6.0 ± 0.4 mmol/l in type 1 diabetic subjects [P = not significant (NS)]. During the final 60 min of each Experimental protocol step, plasma glucose averaged 5.4 ± 0.2, 3.9 ± 0.1, and 3.2 ± 0.1 mmol/l in nondiabetic subjects and 5.6 ± 0.1, 3.9 ± 0.1, and 3.1 ± 0.1 mmol/l in type 1 diabetic subjects (all P = NS between groups at each step). Plasma glucose was maintained at desired targets with coefficients of variation (CVs) for the 5.6, 3.9, and 3.1 mmol/l nominal targets averaging 2.8, 3.4, and 8.7%, respectively, in nondiabetic subjects, and 4.3, 3.7, and 2.2%, respectively, in type 1 diabetic subjects. During Control studies (5.6 mmol/l nominal targets throughout), the CVs were 2.8, 3.0, and 2.2%, respectively, in nondiabetics. Plasma glucose levels during the Control studies averaged 5.4 ± 0.1 mmol/l in the basal period, 5.3 ± 0.2 mmol/l in the first hyperinsulinemic period (corresponding values for each subject required without concomitant glucose infusion, were kept fixed and euglycemia was maintained without concomitant glucose infusion. See text for details. GH, growth hormone.

![Fig. 1. Plasma glucose (mmol/l) and insulin (pmol/l) in Experimental protocols. Data from nondiabetic subjects (□) are compared with those from type 1 diabetic subjects (○). "Basal" refers to study period in which individualized insulin infusions, which each subject required without concomitant glucose infusion, were kept fixed and euglycemia was maintained without concomitant glucose infusion. See text for details. GH, growth hormone.](image-url)
ing to 5.6 mmol/l in the second period (corresponding to 3.9 mmol/l in the Experimental protocol).

After overnight fast (and insulin infusion in the diabetic group), plasma insulin was nearly identical in the two groups, averaging 54 ± 12 pmol/l in nondiabetics and 60 ± 6 pmol/l in diabetics (P = NS). The "basal" individualized insulin infusion rate required to maintain euglycemia during somatostatin infusion was higher in nondiabetics (1.9 ± 0.1 pmol·kg⁻¹·min⁻¹) compared with type 1 diabetics (1.3 ± 0.1 pmol·kg⁻¹·min⁻¹, P < 0.05). During hyperinsulinemia, plasma insulin concentrations in nondiabetics averaged 150 ± 24 pmol/l at the 5.6 mmol/l glucose step, 138 ± 12 pmol/l at the 3.9 mmol/l step, and 126 ± 24 pmol/l at the 3.1 mmol/l step, respectively. As expected, these values were higher than plasma insulin concentrations in diabetics that were 78 ± 18 pmol/l, 84 ± 18 pmol/l, and 78 ± 18 pmol/l at the corresponding time periods (P < 0.05 vs. nondiabetics). In all studies, endogenous insulin secretion in nondiabetics was inhibited by somatostatin such that plasma C-peptide concentrations remained <0.03 (pmol/l) at the detection limit of our assay (not shown).

Glucose infusion rates in the Experimental protocols are shown in Table 1. Under the influence of hyperinsulinemia, the exogenous glucose required to maintain plasma glucose levels was greater in nondiabetics at the 5.6 and 3.9 mmol/l glucose plateaus. Because plasma insulin concentrations were higher in nondiabetics, the greater glucose infusion rate in that group was higher at both euglycemic steps (5.6 mmol/l and 3.9 mmol/l). Plasma glucose-specific activities tended to increase (but not significantly) over the three Experimental periods (Table 1).

Plasma hormone and substrate concentrations. Plasma glucagon (Fig. 2) and GH (Table 2) were clamped at basal concentrations using somatostatin release-inhibiting hormone (SRIH) and replacement infusions throughout all three study periods. Moreover, these levels were not significantly different in the two study groups. Plasma cortisol (Table 2), and epinephrine and norepinephrine (Fig. 2), on the other hand, were free to change. At the 3.9 mmol/l glucose step all three hormones remained at or near basal concentrations and were similar in both diabetic and nondiabetic groups. In particular, plasma epinephrine averaged 453 ± 98 pmol/l in nondiabetics and 267 ± 44 pmol/l in diabetics (P = NS), in neither group exceeding thresholds previously reported to activate EGP (5). However, the reduction of plasma glucose to 3.9 mmol/l from 5.6 mmol/l was associated with an increment in plasma epinephrine in both groups (from 153 ± 33 pmol/l to 267 ± 44 pmol/l in subjects with diabetes, and 142 ± 38 pmol/l to 453 ± 98 pmol/l in nondiabetics, both P < 0.05).

At the final glucose step (3.1 mmol/l), hypoglycemia resulted in significant increments in all three hormones in nondiabetic subjects; average values of plasma cortisol 469 ± 82 nmol/l (P < 0.01), plasma epinephrine 1,823 ± 508 pmol/l (P < 0.001) and plasma norepinephrine 1.91 ± 0.21 nmol/l (P < 0.05) were all significantly increased. Moreover, these values were significantly greater than comparable levels in type 1 diabetics, averaging 248 ± 55 nmol/l (P < 0.05), 961 ± 327 pmol/l (P < 0.02), and 1.39 ± 0.20 nmol/l (P < 0.05), respectively.

Plasma FFA, glycerol and lactate levels during Experimental studies are shown in Table 2. Plasma FFA and glycerol concentrations were stable between the 5.6 mmol/l and 3.9 mmol/l steps in both groups, although the absolute plasma concentrations were generally greater in the type 1 diabetics. Plasma FFA rose significantly (2-fold) during the 3.1 mmol/l step in nondiabetics and tended to increase in type 1 diabetics from the 3.9 to the 3.1 mmol/l steps. Plasma lactate, on the other hand, was unchanged in both groups throughout, although levels at 3.1 mmol/l were higher in the nondiabetic subjects (Table 2).

Glucose turnover. Glucose uptake at baseline (not shown) was 13.8 ± 1.0 µmol·kg⁻¹·min⁻¹ in nondiabetic subjects and 12.5 ± 1.3 µmol·kg⁻¹·min⁻¹ in type 1 diabetics (P = NS). The slightly greater baseline values in the nondiabetics persisted at the hyperinsulinemic 5.6 mmol/l step (Fig. 3), but the difference was more marked with nondiabetics having ~40% higher rates (P < 0.01). When plasma glucose was lowered to 3.9 mmol/l, glucose uptake fell in nondiabetics but was unchanged in diabetics and still remained lower (P < 0.01). Finally, when the 3.1 mmol/l step was achieved, glucose uptake rates in the two groups were nearly identical. Glycolysis from plasma glucose, however, was similar in the two groups at all plasma glucose levels; thus glycogen synthesis accounted for most of the changes observed in glucose uptake (Fig. 3), with rates in diabetics ~30% those of nondiabetics at the 5.6 mmol/l and 3.9 mmol/l steps.

EGP was calculated as the rate of appearance of glucose minus the rate of exogenously infused glucose for the final 60 min of each experimental period. Under basal conditions, EGP was 11.6 ± 0.8 µmol·kg⁻¹·min⁻¹ in the nondiabetic and 11.9 ± 0.9 µmol·kg⁻¹·min⁻¹ in the diabetic subjects, reflecting the objective of the study design to match insulin requirements in the postabsorptive state. When insulin infusion was raised by 20% and plasma glucose was kept stable at the 5.6

Table 1. Glucose infusion rate and [3-³H]glucose specific activity during experimental studies in nondiabetic and type 1 diabetic subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose Step, mmol/l</th>
<th>Glucose Infusion Rate, µmol·kg⁻¹·min⁻¹</th>
<th>Glucose Specific Activity, dpm/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic</td>
<td>5.6</td>
<td>10.9 ± 1.8</td>
<td>1,761 ± 100</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>8.4 ± 1.7</td>
<td>1,913 ± 140</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>3.1 ± 1.2†</td>
<td>1,980 ± 290</td>
</tr>
<tr>
<td>Type 1 diabetic</td>
<td>5.6</td>
<td>3.3 ± 1.1†</td>
<td>2,285 ± 191</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>3.7 ± 0.8†</td>
<td>2,675 ± 233</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>5.4 ± 1.0†</td>
<td>2,765 ± 232</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 compared with value at 5.6 mmol/l step. † P < 0.01 compared with value in nondiabetic subjects at corresponding step.
mmol/l step, EGP was suppressed by an identical ~15% (P < 0.001 for both groups vs. basal, Fig. 4). However, the further reduction of plasma glucose to 3.9 mmol/l resulted in no further decline in EGP in nondiabetics, whereas in the type 1 diabetics EGP fell an additional 25% (P < 0.05 vs. nondiabetics). Also shown in Fig. 4 is the decrease in EGP from basal for each group, indicating that the decrease in EGP in the diabetics at 3.9 mmol/l (4.5 ± 1.0 µmol·kg⁻¹·min⁻¹) was nearly twofold that in nondiabetics of 2.5 ± 0.8 µmol·kg⁻¹·min⁻¹ (P < 0.02). At the final step of frank hypoglycemia (3.1 mmol/l), EGP had returned to near-basal values in nondiabetics but was still profoundly suppressed in diabetic subjects (P < 0.01), indicating a further failure of counterregulation.

To establish whether activation of EGP was indeed present and accounted for the difference seen between the two subject groups at 3.9 mmol/l, we restudied nondiabetics (n = 5) with the Control protocol, which maintained 5.6 mmol/l euglycemia throughout the study. Plasma insulin concentrations in the Experimental and Control protocols, respectively, were 138 ± 12 and 126 ± 6 pmol/l, and plasma glucagon levels were 66 ± 1 and 47 ± 3 ng/l. Under conditions in which plasma

**Table 2.** Counterregulatory hormone and substrate concentrations during experimental studies in nondiabetic and type 1 diabetic subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose Step, mmol/l</th>
<th>Cortisol, nmol/l</th>
<th>GH, µg/l</th>
<th>FFA, µM</th>
<th>Glycerol, µM</th>
<th>Lactate, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic subjects</td>
<td>5.6</td>
<td>193 ± 28</td>
<td>0.8 ± 0.3</td>
<td>87 ± 19</td>
<td>28 ± 5</td>
<td>0.95 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>276 ± 27</td>
<td>0.6 ± 0.1</td>
<td>74 ± 17</td>
<td>29 ± 6</td>
<td>0.87 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>469 ± 82†</td>
<td>0.9 ± 0.2</td>
<td>198 ± 34*</td>
<td>68 ± 20†</td>
<td>1.16 ± 0.16</td>
</tr>
<tr>
<td>Type 1 diabetic subjects</td>
<td>5.6</td>
<td>192 ± 27</td>
<td>0.9 ± 0.1</td>
<td>315 ± 53</td>
<td>48 ± 4†</td>
<td>0.61 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>221 ± 55</td>
<td>0.8 ± 0.1</td>
<td>222 ± 53</td>
<td>41 ± 7</td>
<td>0.56 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>248 ± 54‡</td>
<td>1.2 ± 1.1</td>
<td>297 ± 65</td>
<td>60 ± 12</td>
<td>0.50 ± 0.08‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. GH, growth hormone; FFA, free fatty acids. *P < 0.05 compared with value at 5.6 mmol/l step; †P < 0.01 compared with value at 5.6 mmol/l step; ‡P < 0.01 compared with value in nondiabetic subjects at corresponding step.
glucose and insulin were held stable along with counter-regulatory hormones and plasma FFA (Table 3), rates of EGP fell progressively from basal values comparable to the Experimental protocol (Fig. 5) to values, 40% of basal in the time period corresponding to the 3.9 mmol/l step. At the latter step, EGP during the 5.6 mmol/l Control study was, 50% that during the corresponding period (3.9 mmol/l) of the Experimental study (P, 0.01). This indicates that the effect of the, 1.7 mmol/l difference in plasma glucose (5.6 mmol/l to 3.9 mmol/l) resulted in a difference of, 4.4 µmol·kg\(^{-1}\)·min\(^{-1}\) in EGP; i.e., this change in plasma glucose directly or indirectly antagonized the insulin-induced inhibition of EGP in nondiabetic subjects.

**DISCUSSION**

We have examined the response of EGP to mild hypoglycemia under conditions of hyperinsulinemia together with basal glucagon. The data suggest that the insulin-induced suppression of EGP can be reversed in nondiabetic subjects with unchanged glucagon and minimal changes in epinephrine. Under conditions of euglycemia plus the same degree of hyperinsulinemia in nondiabetics, EGP was 50% more suppressed than when the stimulus of 3.9 mmol/l glucose was present. This early defense against further hypoglycemia may fail in subjects with type 1 diabetes, since EGP at the same mild hypoglycemia was nearly twofold more suppressed than in nondiabetics. Thus we present evidence that glucagon- or epinephrine-independent activation of EGP may be an early step in glucose counterregulation, and this activation is impaired or absent in type 1 diabetes. The present study also confirms the importance of defective counterregulation in type 1 diabetes when plasma glucose levels fall below the normal threshold for epinephrine release (i.e., 3.1 mmol/l). Finally, the data suggest that the effect of
modest hyperinsulinemia to suppress EGP cannot be overcome by intact epinephrine secretion alone, even in nondiabetic subjects at 3.1 mmol/l, and underscore the necessity of intact glucagon responses for the preservation of normal glucose counterregulation.

The mechanism of activation of EGP before and/or independently of the stimulation of glucagon and epinephrine release remains uncertain. The role of glucose per se on hepatic glucose metabolism has been studied primarily under conditions of suppression of EGP by hyperglycemia (2) and is most likely mediated through the modulation of the fluxes through hepatic glucokinase and glucose-6-phosphatase (33). Soskin et al. (46) first proposed that glucose per se regulated glucose output by the liver, although subsequent in vitro studies yielded conflicting results (21, 32, 45). Although not directly pertinent to hypoglycemia, in vitro studies strongly suggest that glucose per se can regulate hepatic glycogen synthesis and breakdown (26).

With reference to hypoglycemia, studies by Saccà et al. (42) indicated that plasma glucagon and epinephrine did not increase significantly during hypoglycemia and low-dose (0.9 pmol·kg⁻¹·min⁻¹) insulin infusion, suggesting the existence of nonhormonal activation of EGP during moderate hypoglycemia in nondiabetic subjects. The interpretation of these data, however, is clouded by 1) lack of any increase in plasma epinephrine or glucagon during frank hypoglycemia that is not consistent with the majority of data from a number of laboratories (9, 35, 43); 2) glucose turnover estimations that used nonpurified [3-³H]glucose without maintenance of stable specific activity; 3) infusion of insulin that was not matched to individual insulin requirements; and 4) glucagon secretion that was not controlled by SRIH.

Bolli et al. (4) addressed some of these deficiencies by using the “islet clamp” technique. However, by use of SRIH to block changes in islet hormones, metyrapone to prevent cortisol secretion, and α- and β-adrenergic blockade to paralyze the sympathetic activation of EGP, they were unable to demonstrate that moderate hypoglycemia contributed any stimulus to overcome insulin-induced suppression of EGP. However, when severe hypoglycemia (plasma glucose ~1.7 mmol/l) was produced, EGP appeared to be activated by hormone-independent mechanism(s). Hansen et al. (23) also suggested that a small (~30%) component of EGP activation could be attributable to the effects of hypoglycemia per se. These conclusions are consistent with our findings, though clearly the study by Bolli et al. was not designed to evaluate the potential role of hormone-independent activation of the liver at mild hypoglycemia. First, the plasma insulin concentrations in their study were 30% higher than ours and their glucagon replacement rates were 35% lower, both of which may have rendered the liver more refractory to stimulation. Second, their studies had other methodological differences (use of nonpurified tracer, no evidence of maintenance of glucose-specific activity, and use of a model of non-steady-state hypoglycemia). Finally, SRIH plus α- and β-adrenergic blockade would, of course, have inhibited both hormonal- and neural-sympathetic activation of EGP. Finally, neither Bolli et al. nor Hansen et al. studied this question in patients with diabetes.

In other nonhuman experimental models, the stimulatory effect of a low glucose concentration per se on glucose oxidations and lipids is well documented. The studies of Bolli et al. (4) and Hansen et al. (23) undoubtedly accounted for this effect. However, in the human studies, the question of whether this stimulation is due to hypoglycemia or hyperinsulinemia or both has not been completely resolved. The results of the present study demonstrate that the effects of hypoglycemia and hyperinsulinemia are additive. The combined stimulatory effects of these two factors may account for a significant portion of the observed increase in glucose production in the diabetic subjects. However, the relative contributions of these two factors require further study.

Table 3. Control studies (euglycemia) in nondiabetic subjects

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.4 ± 0.1</td>
<td>114 ± 6</td>
<td>193 ± 28</td>
<td>0.7 ± 0.1</td>
<td>42 ± 2</td>
<td>120 ± 11</td>
<td>1.1 ± 0.1</td>
<td>301 ± 23</td>
</tr>
<tr>
<td>2</td>
<td>5.3 ± 0.2</td>
<td>126 ± 6</td>
<td>166 ± 27</td>
<td>0.8 ± 0.1</td>
<td>52 ± 1</td>
<td>119 ± 12</td>
<td>1.0 ± 0.1</td>
<td>162 ± 14</td>
</tr>
<tr>
<td>3</td>
<td>5.5 ± 0.2</td>
<td>126 ± 6</td>
<td>221 ± 28</td>
<td>0.7 ± 0.1</td>
<td>47 ± 3</td>
<td>153 ± 11</td>
<td>1.0 ± 0.1</td>
<td>149 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 subjects. Epi, epinephrine; NE, norepinephrine. *Corresponding to 1) basal, 2) 5.6 mmol/l, and 3) 3.9 mmol/l steps in Experimental studies. †P < 0.05 compared with value at step 1.

Table 4. Carbohydrate and lipid oxidation during experimental studies in nondiabetic and type 1 diabetic subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose Oxidation, µmol·kg⁻¹·min⁻¹</th>
<th>µmol·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Step, mmol/l</td>
<td>CHO</td>
</tr>
<tr>
<td>Nondiabetic subjects</td>
<td>5.6</td>
<td>9.4 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>9.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>3.2 ± 2.3†</td>
</tr>
<tr>
<td>Type 1 diabetic subjects</td>
<td>5.6</td>
<td>5.4 ± 1.0†</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>7.0 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>6.1 ± 1.3‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. CHO, carbohydrate. *P < 0.05 compared to value at 5.6 mmol/l step. †P < 0.01 compared to value at 5.6 mmol/l step. ‡P < 0.01 compared with value in nondiabetic subjects at corresponding step.
hepatic glucose output could be distinguished from other potential inputs (e.g., neural). For example, in the perfused rat liver model, sympathetic nerve stimulation enhanced glucose output by activating glycogen phosphorylase activity by a calcium-dependent signaling mechanism (1). This effect may be most directly related to the effect of hypoglycemia because glycogenolysis is probably the first step in counteracting the effects of insulin on the liver (15, 30). Finally, recent studies indicate that the liver may indeed possess an intrinsic ability to sense hypoglycemia via a putative portal vein glucose sensor (25). Neuronal afferents that are sensitive to changes in glucose have been identified in the portal region in nonprimate animal models and have impulse discharges that increase under low glucose conditions (37). The mechanism of the inability of EGP to be activated by mild hypoglycemia in type 1 diabetes is obscure. Given the potential role of hepatic innervation on EGP noted above, one might be tempted to consider this to reflect a form of diabetic autonomic neuropathy. However, our subjects did not exhibit other findings of autonomic neuropathy when crude tests of autonomic function were used. Furthermore, studies in animals with sympathetic denervation mimicking autonomic dysfunction suggest that there is a compensatory hypersensitivity to glucagon and norepinephrine (27). Whether more subtle degrees of autonomic neuropathy in diabetes are responsible for this defect remains a possibility.

Several caveats regarding interpretation of our results are in order. First, the magnitude of the nonhormonal effect on EGP was small and may not be detectable under nonexperimental conditions. Also, although Control (euglycemia) studies in normal subjects were performed to address the question of nonhormonal activation of EGP, we did not perform such a study in DM1 subjects. However, in previous studies using the same pancreatic clamp technique and matching individual basal insulin requirements by exogenous infusion in nondiabetic and type 2 diabetic subjects (33), no time-dependent changes in EGP were noted. We also have been careful to compare nondiabetic with DM1 subjects for the equivalent experimental periods. Second, there is evidence that the central nervous system may directly influence other compensatory counterregulatory mechanisms (e.g., plasma FFA) (34). Plasma FFA concentrations in our studies were greater in subjects with type 1 diabetes and might have explained the difference in glucose uptake, particularly that seen during hypoglycemia (7). Third, there may be other compensating physiological mechanisms for activation of EGP in type 1 diabetes. For example, the liver may be more sensitive to secreted epinephrine in type 1 diabetes (6) independent of the role of hypoinsulinemia (3). In addition, we cannot entirely exclude the possibility that the difference in plasma epinephrine between diabetic and nondiabetic subjects could explain the difference in EGP, although a small increment in plasma epinephrine was seen in both groups. In a previous report (5), levels of plasma epinephrine that were required to significantly increase EGP averaged two- to threefold the levels in our study. Also, these studies do not permit conclusions regarding the potential role of other counterregulatory hormones on early activation of EGP (12, 13), although cortisol and growth hormone are generally thought to play a minor role in the early EGP response (20). Finally, it is possible that the differences in portal insulin concentrations accounted for differential suppression of EGP. In fact, plasma insulin concentrations were higher in nondiabetics compared with type 1 diabetic subjects, and the EGP response in the former may have been underestimated.

These studies in humans are consistent with the body of literature confirming the presence of nonhormonal factors that control EGP in hypoglycemia (36). They lead to several clinical implications of the data. First, a redundant, albeit subtle, mechanism for the early activation of EGP emphasizes the physiological relevance of overlapping counterregulatory mechanisms in protecting the organism from hypoglycemia (20). Second, the lack of an adequate response to a plasma glucose reduction down to 3.9 mmol/l in type 1 diabetes suggests that, in addition to the demonstrated risks of severe hypoglycemia conferred by decreased secretion of glucagon and epinephrine, one must add another component of defective counterregulation that could protect the EGP response.

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REFERENCES


