Blood-to-Brain Glucose Transport, Cerebral Glucose Metabolism, and Cerebral Blood Flow Are Not Increased After Hypoglycemia

Scott A. Segel,[1,3,4] Carmine G. Fanelli,[1,3,4] Carmen S. Dence,[2] Joanne Markham,[2] Tom O. Videen,[2] Deanna S. Paramore,[1,3,4] William J. Powers,[2,5] and Philip E. Cryer[1,3,4]

Recent antecedent hypoglycemia has been found to shift glycemic thresholds for autonomic (including adrenomedullary epinephrine), symptomatic, and other responses to subsequent hypoglycemia to lower plasma glucose concentrations. This change in threshold is the basis of the clinical syndromes of hypoglycemia unawareness and, in part, defective glucose counterregulation and the unifying concept of hypoglycemia-associated autonomic failure in type 1 diabetes. We tested in healthy young adults the hypothesis that recent antecedent hypoglycemia increases blood-to-brain glucose transport, a plausible mechanism of this phenomenon. Eight subjects were studied after euglycemia, and nine were studied after ~24 h of interprandial hypoglycemia (~55 mg/dl, ~3.0 mmol/l). The latter were shown to have reduced plasma epinephrine ($P = 0.009$), neurogenic symptoms ($P = 0.009$), and other responses to subsequent hypoglycemia. Global bihemispheric blood-to-brain glucose transport and cerebral glucose metabolism were calculated from rate constants derived from blood and brain time-activity curves—the latter determined by positron emission tomography (PET)—after intravenous injection of [1-^{11}C]glucose at clamped plasma glucose concentrations of 65 mg/dl (3.6 mmol/l). For these calculations, a model was used that includes a fourth rate constant to account for egress of [^{11}C] metabolites. Cerebral blood flow was measured with intravenous [^{15}O]water using PET. After euglycemia and after hypoglycemia, rates of blood-to-brain glucose transport (24.6 ± 2.3 and 22.4 ± 2.4 μmol · 100 g^{-1} · min^{-1}, respectively), cerebral glucose metabolism (16.8 ± 0.9 and 15.9 ± 0.9 μmol · 100 g^{-1} · min^{-1}, respectively) and cerebral blood flow (56.8 ± 3.9 and 53.3 ± 4.4 ml · 100 g^{-1} · min^{-1}, respectively) were virtually identical. These data do not support the hypothesis that recent antecedent hypoglycemia increases blood-to-brain glucose transport during subsequent hypoglycemia. They do not exclude regional increments in blood-to-brain glucose transport. Alternatively, the fundamental alteration might lie beyond the blood-brain barrier. *Diabetes* 50:1911–1917, 2001

Iatrogenic hypoglycemia is the limiting factor in the glycemic management of diabetes, both conceptually and in practice (1,2). It causes recurrent and sometimes permanent physical morbidity, recurrent or persistent psychosocial morbidity, and occasionally death. It precludes true glycemic control in most patients with type 1 diabetes (3) and many of those with advanced type 2 diabetes (4). Because of the barrier of iatrogenic hypoglycemia, long-term complications of diabetes often develop or progress despite aggressive attempts at glycemic control, albeit at lower rates than during less aggressive therapy (3,4).

At least in type 1 diabetes, iatrogenic hypoglycemia is the result of the interplay of relative or absolute therapeutic insulin excess and compromised physiological and behavioral defenses against developing hypoglycemia (1,2). In established (i.e., C-peptide–negative) type 1 diabetes, circulating insulin levels are unregulated and do not decline as plasma glucose levels decline; the first defense against hypoglycemia is lost. The second defense against developing hypoglycemia, an increase in glucagon secretion in response to falling plasma glucose levels, is also lost in established type 1 diabetes (5,6). Thus, patients with established type 1 diabetes are largely dependent on the third defense against developing hypoglycemia: increased epinephrine secretion. However, that too is often attenuated in type 1 diabetes (6). Those patients with combined deficiencies of their glucagon and epinephrine responses to falling plasma glucose levels have the clinical syndrome of defective glucose counterregulation. Their risk of severe iatrogenic hypoglycemia is ≥25-fold higher than that of patients with absent glucagon but normal epinephrine responses (7,8). Those with reduced epinephrine (i.e., reduced sympathochromaffin) responses also have the clinical syndrome of hypoglycemia unawareness: the loss of warning, largely neurogenic (autonomic) symptoms of developing hypoglycemia, which also often results in episodes of severe hypoglycemia (9).

The concept of hypoglycemia-associated autonomic failure in type 1 diabetes (10–14) posits that because it reduces the autonomic (including adrenomedullary epinephrine as well as sympathetic neural norepinephrine and acetylcholine) and symptomatic responses to a given level of subsequent hypoglycemia, recent antecedent iatrogenic hypoglycemia causes hypoglycemia unawareness.

In addition, because it reduces epinephrine responses in the setting of absent glucagon responses, recent antecedent iatrogenic hypoglycemia also causes defective glucose counterregulation. Perhaps the most compelling support for this concept is the finding in three independent laboratories (14–16) that as little as 2–3 weeks of scrupulous avoidance of iatrogenic hypoglycemia typically reverses hypoglycemia unawareness completely and improves the reduced epinephrine component of defective glucose counterregulation.

The mechanism of hypoglycemia-associated autonomic failure in type 1 diabetes is unknown. The brain glucose transport hypothesis, which posits that recent antecedent hypoglycemia increases blood-to-brain glucose transport during subsequent hypoglycemia, is based on data in rodents and humans. There is substantial evidence that 3–5 days (17–19) or 14 days (20) of hypoglycemia causes increased brain glucose uptake and increased brain microvascular (i.e., blood-brain barrier) GLUT1 mRNA and protein in rats. Boyle et al. (21) reported that 56 h of interprandial hypoglycemia increased brain glucose uptake (determined with the Kety-Schmidt technique) during hypoglycemia in healthy humans and that brain glucose uptake was preserved during hypoglycemia in patients with well-controlled, and frequently hypoglycemic, type 1 diabetes (22). These data suggest that antecedent hypoglycemia causes increased blood-to-brain glucose transport at a given level of subsequent hypoglycemia, a plausible mechanism of hypoglycemia-associated autonomic failure in type 1 diabetes. Accordingly, we measured blood-to-brain glucose transport, along with cerebral glucose metabolism and cerebral blood flow, with positron emission tomography (PET) (23,24) during mild hypoglycemia (65 mg/dl, 3.6 mmol/l) in healthy human subjects after euglycemia and after ~24 h of interprandial hypoglycemia (~55 mg/dl, ~3.0 mmol/l) to test the hypothesis that recent antecedent hypoglycemia causes increased blood-to-brain glucose transport.

RESEARCH DESIGN AND METHODS

A total of 17 healthy young adults gave their informed consent to participate in this study, which was approved by the Washington University Human Studies Committee and conducted at the Washington University General Clinical Research Center (GCRC). Nine subjects (four women, five men) were studied after ~24 h of hypoglycemia (see below). Their age (means ± SD) was 24.9 ± 5.0 years. Eight subjects were studied after ambient euglycemia (three women, five men). Their mean age was 26.6 ± 5.5 years.

Experimental design. Subjects to be studied after hypoglycemia were admitted to the GCRC after an overnight fast early on the morning of day 1. Intravenous lines were inserted into an antecubital vein (for subsequent insulin and glucose infusions) and a contralateral hand vein (with that hand kept in an ~55°C plexiglas box for arterialized venous sampling). Observations (see below) were made at ambient plasma glucose concentrations (~90 mg/dl, ~5.0 mmol/l) from 0600–0700. Then, hyperinsulinemic (2.0 mU · kg^{-1} · min^{-1}, 12.0 pmol · kg^{-1} · min^{-1}) stepped hypoglycemic clamps (25) were performed, and 20% dextrose was infused at variable rates, based on plasma glucose measurements every 5 min, to clamp plasma glucose concentrations at nominal levels of 85 mg/dl (4.7 mmol/l), 75 mg/dl (4.2 mmol/l), 65 mg/dl (3.6 mmol/l), and 55 mg/dl (3.0 mmol/l) in hourly steps. Then, insulin infusions were adjusted to hold plasma glucose concentrations at ~55 mg/dl (~3.0 mmol/l) between meals (lunch, dinner, and snack) until around 0630 on day 2, when glucose levels were raised, and the hyperinsulinemic stepped hypoglycemic clamps were repeated. After the final hypoglycemic step, at 55 mg/dl (3.0 mmol/l), plasma glucose concentrations were clamped at 65 mg/dl (3.6 mg/dl mmol/l), at around 1100, and maintained thereafter. At that point, an arterial sampling line was inserted, and the initial PET studies (see below) were performed. [1-^{11}C]glucose was injected at around 1300.

Subjects to be studied after euglycemia reported to the GCRC after an overnight fast on the morning of the PET studies. Hyperinsulinemic (2.0 mU · kg^{-1} · min^{-1}, 12.0 pmol · kg^{-1} · min^{-1}) mildly hypoglycemic (65 mg/dl, 3.6 mmol/l) clamps were established by 1100, and PET studies (see below) were performed as in the subjects studied after hypoglycemia.

Observations and analytical methods. Arterialized venous plasma glucose concentrations were measured with a glucose oxidase method (Beckman Glucose Analyzer 2; Beckman Instruments, Brea, CA). Arterialized venous samples for other analytes were obtained at 30-min intervals before and during the glucose clamps. Plasma insulin (26), glucagon (27), pancreatic polypeptide (28), growth hormone (29) and cortisol (30) were measured with radioimmunoassay. Plasma epinephrine and norepinephrine were measured with a single isotope derivative (radioenzymatic) method (31). Serum nonesterified fatty acids (32) and blood β-hydroxybutyrate (33), lactate (34), and alanine (35) were measured with enzymatic methods. Symptoms of hypoglycemia were quantitated at 15-min intervals during the glucose clamps by asking the subjects to score (0 = none to 6 = severe) 12 different symptoms, comprised of 6 neurogenic and 6 neuroglycopenic symptoms. The neurogenic symptoms (adrenergic: heart pounding, shaky/tremulous, and nervous/anxious; cholinergic: sweaty, hungry, and tingling) and neuroglycopenic symptoms (difficulty thinking/confused, tired/drowsy, weak, warm, faint, and dizzy) were based on our previously published data (36). Heart rates and blood pressures were recorded automatically (Propaq Encore; Protocol Systems, Beverton, OR) at 15-min intervals, and the ECG was monitored throughout the hypoglycemic clamps.

PET methods. Global bihemispheric rates of blood-to-brain glucose transport and cerebral glucose metabolism were calculated from rate constants derived from blood and brain time-activity curves (the latter determined by PET) after intravenous injection of [1-^{11}C]glucose using a model that includes a fourth rate constant to account for egress of [^{11}C]metabolites (23,24). Bihemispheric cerebral blood volume was measured with inhaled [^{15}O]carbon monoxide (37,38), and bihemispheric cerebral blood flow was measured with intravenously injected [^{15}O]water (37,39), also using PET. These methods have been previously described in detail (23,24,37–39). The [1-^{11}C]glucose PET method has been validated in macaques, in which is was shown that net brain glucose extraction measured with the PET method was correlated with that determined by arteriovenous differences; it was also shown that blood-to-brain glucose transport was related to arterial glucose extraction using appropriate Michaelis-Menten kinetics (23). This method was also applied in an earlier study in humans (24). More recently, we have shown that the rates of blood-to-brain glucose transport and cerebral glucose metabolism determined with the PET method are unaffected by circulating radiolabeled nonglucose metabolites (40). Blood-to-brain glucose transport determined with this method has been shown to vary as a function of the plasma glucose concentration (23,41).

Statistical methods. Data in this manuscript are reported as the means ± SE, except where SD is specified. Data from the hyperinsulinemic stepped hypoglycemic clamps were analyzed by repeated-measures analysis of variance. Those from the PET studies were analyzed by t test. P values < 0.050 were considered to indicate statistically significant differences.

RESULTS

Effects of antecedent hypoglycemia. Hourly plasma glucose concentrations (mean ± SE), including those after lunch, dinner, and a snack, were 64 ± 1 mg/dl (3.6 ± 0.1 mmol/l) between the stepped hypoglycemic clamps on day 1 and the clamps on day 2 in the subjects studied after hypoglycemia (Figs. 1-4, Table 1). During the hyperinsulinemic stepped hypoglycemic clamps, plasma insulin concentrations were raised comparably, and target plasma glucose concentrations (85, 75, 65, and 55 mg/dl; 4.7, 4.2, 3.6, and 3.0 mmol/l, respectively) were achieved on days 1 and 2 (Fig. 1). Compared with the responses on day 1, plasma epinephrine responses to hypoglycemia were reduced ($P = 0.009$) on day 2 after interval hypoglycemia; plasma norepinephrine responses were not reduced significantly ($P = 0.290$) (Fig. 2). Compared with the responses on day 1, neurogenic (autonomic) symptom score responses to hypoglycemia were reduced ($P = 0.009$) on day 2 after interval hypoglycemia; neuroglycopenic symptom score responses were not reduced significantly ($P = 0.130$)
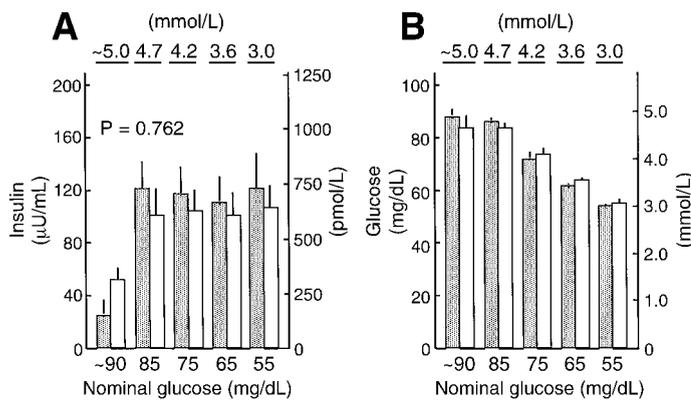
FIG. 1. Mean ± SE plasma values for insulin (*A*) and glucose (*B*) concentrations before and during hyperinsulinemic stepped hypoglycemic clamps before (day 1, ■) and after (day 2, □) ~24 h of hypoglycemia (55 mg/dl, 3.0 mmol/l) in nine subjects who underwent PET studies after hypoglycemia.

FIG. 2. Mean ± SE values for plasma epinephrine (*A*) and norepinephrine (*B*) concentrations before and during hyperinsulinemic stepped hypoglycemic clamps before (day 1, ■) and after (day 2, □) ~24 h of hypoglycemia (55 mg/dl, 3.0 mmol/l) in nine subjects who underwent PET studies after hypoglycemia.

(Fig. 3). Compared with the responses on day 1, plasma glucagon responses to hypoglycemia were reduced ($P = 0.031$) on day 2 after interval hypoglycemia; plasma pancreatic polypeptide responses were not reduced significantly ($P = 0.134$) (Fig. 4). Compared with the responses on day 1, plasma growth hormone responses to hypoglycemia were reduced ($P = 0.001$) on day 2 after interval hypoglycemia; plasma cortisol responses were not reduced significantly ($P = 0.087$) (Table 1). Serum nonesterified fatty acid levels and blood β-hydroxybutyrate and alanine levels were similar on days 1 and 2 (Table 1); blood lactate responses were reduced on day 2 (Table 1).

PET studies after hypoglycemia and after euglycemia. During the PET studies, plasma arterial glucose concentrations were clamped at comparable mildly hypoglycemic levels of 66 ± 1 mg/dl (3.7 ± 0.1 mmol/l) in the nine subjects studied after hypoglycemia and 64 ± 0 mg/dl (3.6 ± 0.0 mmol/l) in the eight subjects studied after
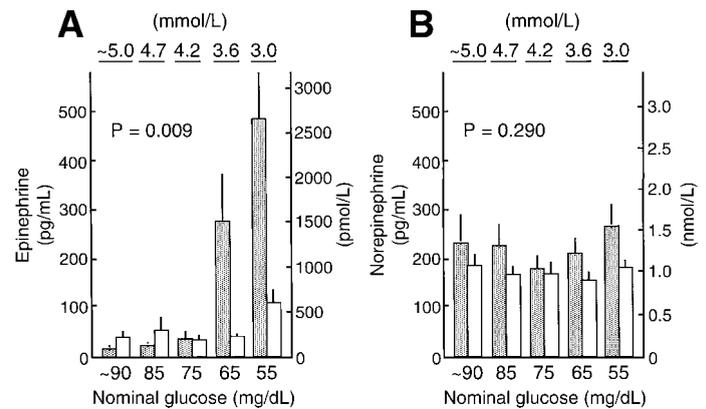
euglycemia (Table 2, Fig. 5). The circulating levels of neuroendocrine parameters were lower, or tended to be lower, in the subjects studied after hypoglycemia compared with those studied after euglycemia (Table 2).

Global bihemispheric rates of blood-to-brain glucose transport (24.6 ± 2.3 vs. 22.4 ± 2.4 μmol · 100 g^{-1} · min^{-1} after euglycemia and after hypoglycemia, respectively), cerebral glucose metabolism (16.8 ± 0.9 vs. 15.9 ± 0.9 μmol · 100 g^{-1} · min^{-1} after euglycemia and after hypoglycemia, respectively), and cerebral blood flow (56.8 ± 3.9 vs. 53.3 ± 4.4 ml · 100 g^{-1} · min^{-1} after euglycemia and after hypoglycemia, respectively) were virtually identical under the two study conditions (Fig. 5). In addition, cerebral blood volumes (3.63 ± 0.11 vs. 3.25 ± 0.14 ml/100 g after euglycemia and after hypoglycemia, respectively) were similar. Based on calculations of the confidence intervals for the differences between the groups, the proba-

TABLE 1
Plasma growth hormone, cortisol, serum nonesterified fatty acids, blood β-hydroxybutyrate, lactate, and alanine concentrations before and during hyperinsulinemic stepped hypoglycemic clamps before (Day 1) and after (Day 2) approximately 24 h of hypoglycemia (55 mg/dl, 3.0 mmol/l) in nine subjects who underwent PET studies after hypoglycemia

	Nominal glucose (mg/dl)					
	~90	85	75	65	55	*P*
Growth hormone (ng/ml)*						
Day 1	1.3 ± 0.7	1.5 ± 1.0	2.1 ± 1.5	7.7 ± 2.1	18.2 ± 2.4	0.001
Day 2	1.1 ± 0.4	3.5 ± 2.4	5.1 ± 2.4	2.6 ± 1.3	8.4 ± 2.8	
Cortisol (μg/dl)†						
Day 1	11.5 ± 1.6	10.3 ± 1.7	8.9 ± 1.1	12.5 ± 1.6	17.1 ± 1.8	0.087
Day 2	13.4 ± 1.8	9.8 ± 1.7	12.4 ± 3.1	9.8 ± 2.0	17.4 ± 3.6	
Nonesterified fatty acids (μmol/l)						
Day 1	381 ± 76	104 ± 26	79 ± 14	79 ± 20	66 ± 22	0.592
Day 2	307 ± 84	74 ± 8	54 ± 7	48 ± 10	45 ± 10	
β-Hydroxybutyrate (μmol/l)						
Day 1	145 ± 37	64 ± 24	106 ± 38	66 ± 24	66 ± 17	0.927
Day 2	111 ± 22	86 ± 22	64 ± 18	84 ± 28	68 ± 19	
Lactate (μmol/l)						
Day 1	1045 ± 129	1451 ± 163	1349 ± 95	1308 ± 81	1824 ± 231	0.001
Day 2	963 ± 115	1024 ± 68	839 ± 90	753 ± 69	1063 ± 94	
Alanine (μmol/l)						
Day 1	469 ± 54	407 ± 26	420 ± 421	377 ± 46	403 ± 33	0.342
Day 2	291 ± 34	311 ± 26	310 ± 31	257 ± 27	259 ± 26	

Data are means ± SE. *To convert growth hormone to pmol/l, multiply by 44.15; †to convert cortisol to nmol/l, multiply by 27.59.
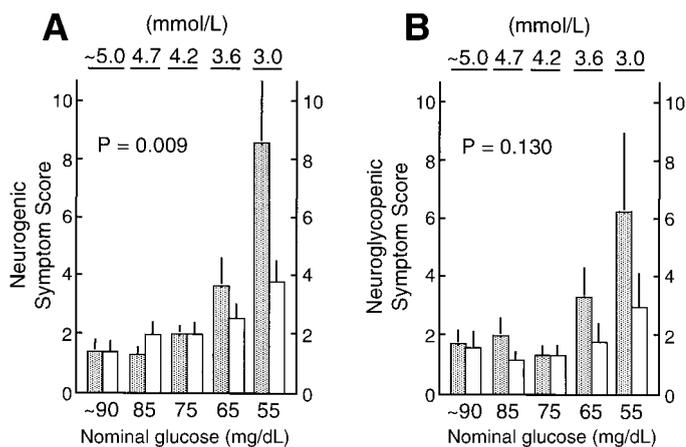
FIG. 3. Mean ± SE values for neurogenic (*A*) and neuroglycopenic (*B*) symptom scores before and during hyperinsulinemic stepped hypoglycemic clamps before (day 1, ■) and after (day 2, □) ~24 h of hypoglycemia (55 mg/dl, 3.0 mmol/l) in nine subjects who underwent PET studies after hypoglycemia.

bility is <5% that blood-to-brain glucose transport was increased ≥20%, cerebral glucose metabolism was increased by ≥10%, or cerebral blood flow was increased by ≥16% after hypoglycemia.

DISCUSSION

These data do not support the hypothesis that recent antecedent hypoglycemia causes increased blood-to-brain glucose transport (17–22). Rates of blood-to-brain glucose transport, as well as those of cerebral glucose metabolism and cerebral blood flow (determined with radiotracers and PET) were virtually identical in healthy subjects studied after euglycemia and in those studied after ~24 h of interprandial hypoglycemia (~55 mg/dl, ~3.0 mmol/l). The mean plasma glucose level, including excursions after lunch, dinner, and a bed time snack, was 64 ± 1 mg/dl (3.6 ± 0.1 mmol/l). Although we cannot categorically exclude small differences (given our sample size), there was no trend in blood-to-brain glucose transport, cerebral glucose metabolism, or cerebral blood flow in the direction of our hypothesis. Indeed, the means of all three

FIG. 4. Mean ± SE values for plasma glucagon and pancreatic polypeptide concentrations before and during hyperinsulinemic stepped hypoglycemic clamps before (day 1, ■) and after (day 2, □) ~24 h of hypoglycemia (55 mg/dl, 3.0 mmol/l) in nine subjects who underwent PET studies after hypoglycemia.

TABLE 2
Neuroendocrine parameters during the PET studies in eight subjects studied after euglycemia and nine subjects studied after approximately 24 h of hypoglycemia*

	After EU	After HYPO	*P*
Glucose (mg/dl)	64 ± 0	66 ± 1	—
Epinephrine (pg/ml)	302 ± 77	112 ± 34	0.032
Norepinephrine (pg/ml)	253 ± 29	208 ± 31	0.310
Glucagon (pg/ml)	118 ± 29	43 ± 8	0.012
Pancreatic polypeptide (pg/ml)	117 ± 35	38 ± 9	0.035
Growth hormone (ng/ml)	12.1 ± 4.8	10.6 ± 4.1	0.809
Cortisol (μg/dl)	14.2 ± 2.4	11.7 ± 2.1	0.430

Data are means ± SE. *To convert glucose to mmol/l multiply by 0.0551, epinephrine to pmol/l multiply by 5.485, norepinephrine to nmol/l multiply by 0.005911, glucagon to pmol/l multiply by 0.2871, pancreatic polypeptide to pmol/l multiply by 0.239, growth hormone to pmol/l multiply by 44.15, and cortisol to nmol/l multiply by 27.59. EU, euglycemia; HYPO, hypoglycemia.

variables tended to be lower after hypoglycemia. Analysis of the differences between the groups disclosed that there is a <5% statistical probability that after hypoglycemia, blood-to-brain glucose transport was increased by 20%, which is the increase Boyle et al. (21) estimated was required in order to explain their findings. Similarly, there is a <5% probability that after hypoglycemia, cerebral blood flow was increased by 16%, which is approximately half the increase reported by Boyle et al. (21).

Because measurements of labeled glucose in brain were made shortly after intra-arterial injection of labeled glucose, the data indicating that 3–5 (17–19) or 14 (20) days of hypoglycemia increases brain glucose uptake in rodents largely reflect an increase in unidirectional blood-to-brain glucose transport. On the other hand, because the Kety-Schmidt technique measures net glucose uptake by the brain, the data indicating that 56 h of interprandial hypoglycemia (21) or frequent iatrogenic hypoglycemia in type 1 diabetes (22) increases brain glucose uptake during hypoglycemia largely reflect an increase in brain glucose metabolism. We measured blood-to-brain glucose transport and cerebral glucose metabolism in human subjects
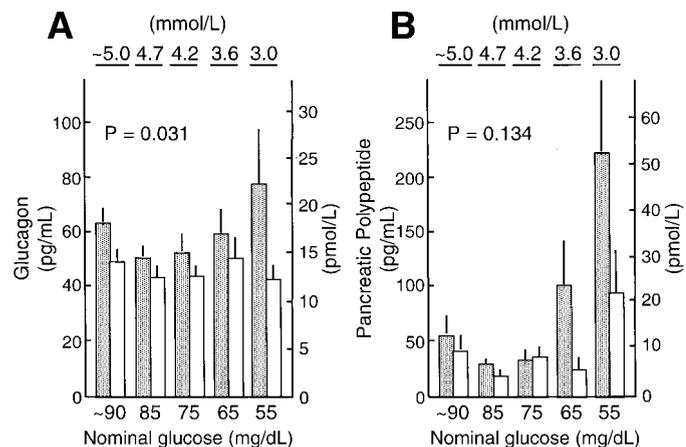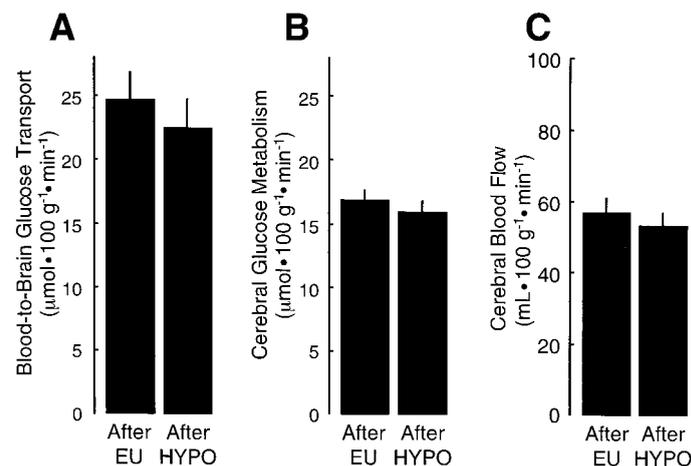

FIG. 5. Mean ± SE rates of blood-to-brain glucose transport (*A*), cerebral glucose metabolism (*B*), and cerebral blood flow (*C*) determined with PET during hyperinsulinemic mildly hypoglycemic clamps (65 mg/dl, 3.6 mmol/l) in eight subjects studied after euglycemia (After EU) and nine subjects studied after ~24 h of hypoglycemia (55 mg/dl, 3.0 mmol/l) (After HYPO).

with a different method: [1-^{11}C]glucose and PET (23). This method has been validated in macaques, in which blood-to-brain glucose transport was shown to be related to arterial plasma glucose extraction by appropriate Michaelis-Menten kinetics, and net glucose extraction measured with the PET method correlated with that determined by arteriovenous glucose differences (23). It has also been applied to an earlier study in humans showing that neither blood-to-brain glucose transport nor cerebral glucose metabolism is reduced in poorly controlled type 1 diabetes (24). It differs from other PET methods (42–44) in that it uses both [^{11}C]glucose labeled in the C-1 position and a model that includes a fourth rate constant to account for the egress of [^{11}C]metabolites (23). We have determined that the calculated rates of blood-to-brain glucose transport and cerebral glucose metabolism determined with this method are unaffected by circulating radiolabeled nonglucose metabolites (40) and that blood-to-brain glucose transport varies as a function of the plasma glucose concentration (23,41).

In the subjects who underwent PET studies after hypoglycemia, glycemic thresholds for autonomic, symptomatic, and other responses to hypoglycemia were shifted to lower plasma glucose concentrations (i.e., lower plasma glucose levels were required to elicit these responses) after interval hypoglycemia, as expected (10–14,45,46). During hyperinsulinemic stepped hypoglycemic clamps, plasma epinephrine, neurogenic symptom scores, and plasma glucagon responses were reduced significantly, and plasma norepinephrine, neuroglycopenic symptom scores, and pancreatic polypeptide responses tended to be reduced after ~24 h of interprandial hypoglycemia. These are fundamental features of the concept of hypoglycemia-associated autonomic failure in type 1 diabetes (10–14) that we sought to explain mechanistically. If these threshold shifts were the result of increased blood-to-brain glucose transport, and thus of the provision of more glucose to the brain with correspondingly reduced responses during subsequent hypoglycemia, rates of blood-to-brain glucose transport should have been higher in these subjects than in those studied in the absence of antecedent hypoglycemia. However, that was not the case.

In the studies of Boyle et al. (21,22), using the Kety-Schmidt technique, the higher calculated rates of brain glucose uptake during hypoglycemia after 56 h of interprandial hypoglycemia in healthy subjects (21) and in patients with type 1 diabetes and HbA$_{1c}$ values in the lowest tertile (22) were a function of both higher rates of cerebral blood flow and larger glucose arteriovenous differences across the brain. Cerebral blood flow, measured with the nitrous oxide method, was higher after interprandial hypoglycemia at clamped plasma glucose concentrations as high as 65 mg/dl (3.6 mmol/l) in the former study (21). Indeed, in that study at the 65 mg/dl plasma glucose step, the calculated ~40% higher rate of brain glucose uptake was entirely attributable to an ~40% higher cerebral blood flow because the glucose arteriovenous difference was not higher. At the 55 mg/dl (3.0 mmol/l) glucose step, cerebral blood flow was ~30–35% higher, whereas the glucose arteriovenous difference was only ~5–10% higher. In contrast, with regard to the rates of cerebral blood flow (measured with [^{15}O]water and PET)

after ~24 h of hypoglycemia, we found no difference compared with the rates after euglycemia.

In theory, a difference in blood-to-brain glucose transport under these two study conditions would be demonstrable at any plasma glucose concentration, provided the glucose levels were comparable at the time of the measurements. However, we elected to perform the PET measurements at the mildly hypoglycemic level of 65 mg/dl (3.6 mmol/l). That approximates the plasma glucose concentration at which brain glucose uptake has been reported to begin to decline (21) and is slightly below the normal glycemic thresholds for glucose counterregulatory hormone secretion of ~65–68 mg/dl (3.6–3.8 mmol/l) (2,25,47,48). Notably, for plasma epinephrine, pancreatic polypeptide, and glucagon concentrations during this mild hypoglycemia at the time of the PET measurements in the subjects studied after interval hypoglycemia, levels were lower than in the subjects studied after euglycemia, providing additional evidence of the phenomenon we sought to explain mechanistically. Nonetheless, we found no effect of antecedent hypoglycemia on blood-to-brain glucose transport (or on cerebral glucose metabolism or cerebral blood flow) under this clinically relevant condition.

We studied the effect of only ~24 h of antecedent interprandial hypoglycemia and raised plasma glucose levels transiently to 85 mg/dl (4.7 mmol/l) (to repeat the hyperinsulinemic stepped hypoglycemic clamps) the morning before performing the PET studies on day 2 in the subjects studied after hypoglycemia. Thus, the duration of antecedent hypoglycemia was shorter than that in the earlier rodent studies (17–20) and one of the earlier human studies (21), and unlike the rodent studies (17–20), glucose levels were raised before the PET studies. However, even if our findings were different after a longer period of antecedent hypoglycemia, and even if plasma glucose levels were not raised temporarily before the PET studies, those findings would not be relevant to the clinical phenomenon we sought to explain mechanistically, i.e., elevated glycemic thresholds for autonomic (including epinephrine) and symptomatic responses after relatively brief episodes of iatrogenic hypoglycemia in people with diabetes.

The subjects studied after euglycemia, unlike those studied after hypoglycemia, were not subjected to hyperinsulinemia the previous day. However, antecedent hyperinsulinemic euglycemia, in contrast to hypoglycemia, does not reduce responses to hypoglycemia the following day (2,45). This is the phenomenon that we sought to explain mechanistically and that we documented in the present study. Furthermore, all of the PET studies were performed under hyperinsulinemic conditions. However, hyperinsulinemia per se does not increase blood-to-brain glucose metabolism or cerebral glucose metabolism (49,50).

Our data do not support the hypothesis that recent antecedent hypoglycemia causes increased global blood-to-brain glucose transport, but they do not exclude regional increments in blood-to-brain glucose transport. However, the published data in rodents (17–20) and humans (21,22) that provided the basis of the hypothesis we tested included measures of global, rather than regional, brain glucose transport and metabolism, respectively. Nonetheless, it is conceivable that regional increments in blood-to-brain glucose transport (e.g., in the ventromedial

hypothalamus, among other sites) might explain the phenomenon of hypoglycemia-associated autonomic failure in diabetes. Alternatively, the fundamental alteration might lie beyond the blood-brain barrier (51,52).

ACKNOWLEDGMENTS

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