Journal of Neuroscience Research 79:42–47 (2005)

Brain Glucose Concentrations in Patients with Type 1 Diabetes and Hypoglycemia Unawareness

Amy B. Criego,[1]* Ivan Tkac,[3] Anjali Kumar,[2] William Thomas,[5] Rolf Gruetter,[3,4] and Elizabeth R. Seaquist[2]

[1]Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota
[2]Department of Medicine, University of Minnesota Medical School, Minneapolis, Minnesota
[3]Department of Radiology, University of Minnesota Medical School, Minneapolis, Minnesota
[4]Department of Neuroscience, University of Minnesota Medical School, Minneapolis, Minnesota
[5]Division of Biostatistics, University of Minnesota School of Public Health, Minneapolis, Minnesota

Although it is well established that recurrent hypoglycemia leads to hypoglycemia unawareness, the mechanisms responsible for this are unknown. One hypothesis is that recurrent hypoglycemia alters brain glucose transport or metabolism. We measured steady-state brain glucose concentrations during a glucose clamp to determine whether subjects with type 1 diabetes and hypoglycemia unawareness may have altered cerebral glucose transport or metabolism after exposure to recurrent hypoglycemia. We compared 14 subjects with diabetes and hypoglycemia unawareness to 27 healthy control subjects. Brain glucose concentrations were measured under similar metabolic conditions using in vivo ^{1}H nuclear magnetic resonance (NMR) spectroscopy at 4 Tesla during a hyperglycemic clamp (plasma glucose = 16.7 mmol/l) with somatostatin and insulin. Subjects with type 1 diabetes and hypoglycemia unawareness had significantly higher brain glucose concentrations compared to that in controls under the same conditions (5.5 ± 0.3 vs. 4.7 ± 0.1 μmol/g wet weight, $P = 0.016$). These data suggest that changes in brain glucose transport or metabolism may occur as a result of recurrent hypoglycemia.

Key words: hypoglycemia unawareness; type 1 diabetes; magnetic resonance spectroscopy; brain glucose

Hypoglycemia remains the limiting factor for achieving normal glycemic control in patients with type 1 diabetes (Cryer, 1994). The findings of the Diabetes Control and Complications Trial (DCCT) established that improved glucose control significantly reduces the rate at which diabetes-related complications occur in subjects with type 1 diabetes (DCCT Research Group, 1993). It also determined, however, that patients with type 1 diabetes and hemoglobin A1c values less than 7.5% experience hypoglycemia three times more often than do those with poor control (DCCT Research Group, 1993). The fear associated with an increased frequency of hypoglycemia is a major barrier in intensifying insulin management for the purpose of preventing the long-term complications of diabetes.

Recurrent hypoglycemia frequently leads to the condition of hypoglycemia unawareness in which a patient is no longer able to perceive symptoms of hypoglycemia and consequently is unable to take action to prevent the development of severe hypoglycemia. In these cases, the autonomic symptoms of hypoglycemia (tachycardia, nervousness, and tremor) are not recognized or do not occur before development of neuroglycopenia. Intensively treated patients with type 1 diabetes require a lower glucose level to elicit a counterregulatory response than do more poorly controlled patients (Amiel et al., 1988; Dagogo-Jack et al., 1993; Cryer, 1994). The mechanism underlying this condition, which has been termed hypoglycemia-associated autonomic failure (HAAF), is unclear but may involve a compensatory increase in cerebral glucose transport rate so that sufficient glucose is provided to the brain for its energy needs even in the face of systemic hypoglycemia (Boyle et al., 1994).

Traditional thinking is that a continuous supply of glucose is necessary for normal brain function with the brain being unable to store more than a few minutes worth of glucose for use during hypoglycemia (Pardridge, 1983). Glucose reaches the central nervous system (CNS) by crossing the blood–brain barrier via the specific transport protein, GLUT1 (Pardridge et al., 1990). When exposed to hypoglycemia, transcription and translation (Boado and Pardridge 1993) of endothelial GLUT1 at the blood–brain barrier are increased. Chronic and prolonged hypoglycemia in rodents increased the number of glucose transporters in the brain (Koranyi et al., 1991), resulting in increased movement of glucose (McCall et al., 1982). In

Contract grant sponsor: National Institutes of Health; Contract grant number: RO1-NS35192, MO1 RR00400, P41 RR08079; Contract grant sponsor: Juvenile Diabetes Research Foundation; Contract grant number: 13-2002-445.

*Correspondence to: Amy B. Criego, MD, MMC 404, 420 Delaware St. SE, Minneapolis, MN 55455. E-mail: crieg002@umn.edu

Received 9 July 2004; Revised 4 August 2004; Accepted 6 August 2004

Published online 2 December 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.20296

patients with frequent hypoglycemia and near normal values of hemoglobin A1c (7.2 ± 0.5%), brain glucose uptake in the face of systemic hypoglycemia is unchanged from euglycemia, whereas patients with poorly controlled diabetes and healthy controls have reduced brain glucose uptake during systemic hypoglycemia (Boyle et al., 1995). These observations suggest that brain glucose uptake may be increased in response to recurrent hypoglycemia in patients with well-controlled diabetes, therefore causing an increase in brain glucose concentration to ensure an adequate delivery of substrate during subsequent episodes of hypoglycemia. This compensatory increase in brain glucose concentration may be partly responsible for the inability to detect symptoms of hypoglycemia in patients with hypoglycemia unawareness. Studies with positron emission tomography (PET) have been unable to support this hypothesis, although none were sufficiently powered to identify metabolic differences that were smaller than 20% (Segel et al., 2001).

Although other methods have been used to examine brain glucose metabolism, proton magnetic resonance (MR) spectroscopy is currently the only method that allows quantification of in vivo native glucose concentrations. With current methodology, sufficient signal-to-noise to permit good resolution of the glucose signal and accurate quantification of brain glucose concentrations is obtained only under hyperglycemic conditions (Gruetter, 1996). We have demonstrated previously that a linear relationship exists between plasma and brain glucose concentrations with plasma glucose values ranging from 4.4–24.5 mmol/l in humans (Seaquist et al., 2001). Similar observations have been made by Choi et al. (2001) in animals with plasma glucose values ranging from 0.2–30 mmol/l. We can therefore anticipate that differences in brain glucose concentrations detected during hyperglycemia would also exist during hypoglycemia.

To determine whether recurrent hypoglycemia in patients with type 1 diabetes results in small but presumably clinically relevant changes in brain glucose metabolism, we measured steady-state brain glucose concentrations under controlled metabolic conditions using high-field ^{1}H MR spectroscopy. We hypothesized that subjects with hypoglycemia unawareness would have higher brain glucose concentrations than control subjects would under these conditions and that our observations would provide insights into the effect of recurrent hypoglycemia on brain glucose transport and metabolism.

MATERIALS AND METHODS

Study Participants

Subjects with intensively treated type 1 diabetes of greater than 5 years duration were recruited from the Endocrine Clinics at the University of Minnesota. To be included, subjects were required to be ≥18 years of age, have a hemoglobin A1c ≤7.5% at the time of recruitment (normal subject range was 4.5–6.0%), experience a minimum of two self-reported episodes of hypoglycemia per week, and have hypoglycemia unawareness verified by the questionnaire developed by Clarke et al. (1995). This questionnaire consists of eight questions about the frequency and severity of asymptomatic hypoglycemia (defined as home glucose readings <70 mg/dl) and severe hypoglycemia (defined as episodes in which the subjects was unconscious or had a seizure and needed glucagon or intravenous glucose for recovery), and has been validated as a reliable method to identify subjects with type 1 diabetes and hypoglycemia unawareness (Clarke et al., 1995). In addition to these criteria, subjects also met the requirements for the MR study, which excludes subjects weighing more than 300 pounds. Control subjects were recruited from the Fairview-University Medical Center and University of Minnesota communities and were similar to the subjects with type 1 diabetes and hypoglycemia unawareness with respect to body mass index and gender. The study protocol was conducted according to procedures approved by the Institutional Review Board at the University of Minnesota.

Protocol

Subjects with type 1 diabetes were asked to monitor and record their blood glucose values before bedtime and at each meal during the week before the study. The subjects with type 1 diabetes were admitted to the General Clinical Research Center at the University of Minnesota the night before the experiment. Their evening dose of long-acting insulin was withheld and they were maintained on an intravenous infusion of insulin overnight (approximately 8–10 hr) to maintain their blood glucose between 5.6–10.0 mmol/l (100–180 mg/dl). At 7:30 AM the following morning, the insulin infusion was discontinued and subjects were transported to the Center for Magnetic Resonance Research for the experiment.

All subjects were studied in the morning at the Center for Magnetic Resonance Research after an overnight fast. In preparation for the experiment, an intravenous catheter was placed in each in each antecubital region for the delivery of glucose, insulin, potassium, and somatostatin. A third intravenous catheter was placed retrograde in the foot for blood sampling. This leg was wrapped in heated towels and hot packs to arterialize the venous blood (Seaquist, 1997). At baseline, samples were obtained for glucose, insulin, and ketones. The somatostatin infusion was begun and advanced to a rate of 0.16 μg/kg/min over 30–45 min to suppress endogenous insulin secretion (Yen et al., 1974). An intravenous insulin infusion (0.5 mU/kg/min, Humulin Regular; Eli Lilly and Company) was started in conjunction with the somatostatin at time zero. During the first 30–45 min, basal euglycemia was maintained by infusing glucose (50% dextrose) at a variable rate while the somatostatin infusion was increased. Thereafter, the glucose infusion was increased rapidly to clamp the plasma glucose concentration at 16.7 mmol/l; a level sufficiently elevated to yield a robust signal of the H-1 proton of the α-glucose at 5.23 ppm in spectra acquired from the human brain. Spectroscopic measurements were begun after subjects had been at stable hyperglycemia for a minimum of 20 min. The study design is depicted in Figure 1.

MR Spectroscopy

Experiments were carried out on a 4-Tesla Siemens/Oxford magnet interfaced to a Varian console as described previously (Gruetter et al., 1998a,b). Subjects were placed supine on a bed above the surface coil and their heads were held in place by cushions. To decrease the exposure to gradient noise,

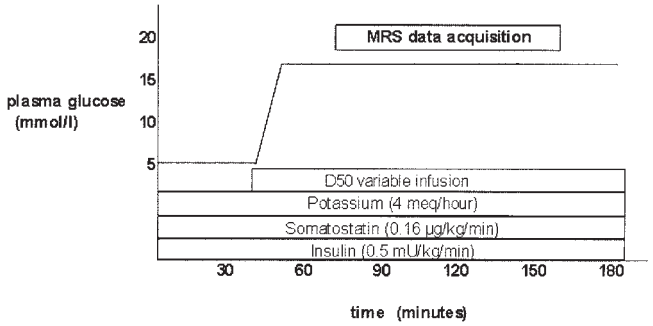

Fig. 1. Experimental protocol. Subjects were given intravenous infusions of somatostatin and insulin starting at time zero. Glucose was then infused as necessary to maintain plasma glucose at 16.7 mmol/l between 0–180 min. Data to measure cerebral glucose concentrations were acquired by MR spectroscopy during the final 60 min of the study.

all subjects wore earplugs. A quadrature transmit/receive half-volume radio frequency (RF) coil was used (Adriany and Gruetter, 1997). A 16-ml volume of brain in the occipital cortex was identified for data acquisition. Selection of the volume of interest (VOI) for the spectroscopy was based on anatomic landmarks identified on T2-weighted rapid acquisition with relaxation enhancement (RARE) multislice magnetic resonance imaging (MRI; Lee et al., 1995). In vivo ^{1}H MR spectra were measured using short echo-time stimulated echo acquisition mode (STEAM) (echo time [TE] = 4 msec; repetition time [TR] = 4.5 sec) combined with outer volume suppression and variable power and optimized relaxation delays (VAPOR) water suppression (Tkac et al., 1999). The spectra from the VOI were measured in an interleaved manner. Localized shimming was carried out using FASTMAP (Gruetter, 1993; Gruetter and Tkac, 2000), which has resulted in consistent linewidths for the water resonance of 7–9 Hz. Data were averaged over a period of 1.2 min (blocks of 16 scans), which were stored separately in memory. This allowed elimination of small frequency drifts between spectra before summation on the order of a few hertz and averaged over at least 10 min. In each case, the plasma glucose concentration was maintained at steady state for at least 20 min before spectral data were acquired. Data from the VOI was acquired over a minimum period of 25 min during which glycemia was clamped at the target level.

Nuclear MR Spectral Analysis

Free induction decays were zero filled and apodized with exponential multiplication (2 Hz line broadening). An example of the spectra acquired is shown in Figure 2. Peak area of the glucose (at 5.23 ppm), creatine (3.03 ppm), and macromolecule (2.98 ppm) resonances were quantified using peak-fitting software supplied by the spectrometer manufacturer, as described previously (Gruetter et al., 1996, 1998a). The area under the glucose peak at 5.23 ppm was calculated relative to the area under the creatine methyl resonance at 3.04 ppm. The concentration of creatine was set to 10 μmol/g wet weight based on cortical concentrations of 9.6 μmol/g wet weight (Petroff et al., 1989) and on contributions of 1 μmol/g wet weight γ-aminobutyric acid (GABA; Rothman et al., 1993) and 1–2 μmol/g wet weight glutathione (GSH) in this region of the brain (Choi et al., 2002; Terpstra et al., 2002).

Assessment of Hormonal Counterregulation

Subjects with type 1 diabetes and hypoglycemia unawareness were asked to undergo an assessment of hormonal counterregulation to hypoglycemia within 24 hr of completing the magnet study. In this assessment, insulin was infused intravenously at a rate of 2.0 mU/kg/min and blood glucose was allowed to fall to 2.7 mmol/l, where it was maintained for 30 min. Samples for the immediate measurement of plasma glucose were obtained every 5 min and glucose (20% dextrose) was infused intravenously as necessary to maintain the glucose at the target level. Samples for the subsequent measurement of glucagon were collected at baseline and every 10 min during hypoglycemia in iced tubes containing EDTA/Trasylol (500 U/ml). Samples collected for subsequent measurement of epinephrine and norepinephrine were collected using the same schedule in chilled tubes containing 50 μl of a solution of EGTA (90 mg/ml) and GSH (60 mg/ml). Counterregulation was assessed similarly in a group of control subjects for comparison.

Laboratory Analyses

During the study, blood samples were obtained every 5 min for determination of the blood glucose concentration using an autoanalyzer (Beckman, Fullerton, CA). During the magnet study, additional samples were taken every 30 min for later determination of serum insulin concentrations by a Beckman Access instrument. Serum ketones were measured in the clinical laboratory using a qualitative test based on a nitroferrocyanide reaction. Hemoglobin A1c was measured in the subjects with type 1 diabetes by high-performance liquid chromatography (HPLC; DCCT Research Group, 1987). Blood samples obtained during the hypoglycemia study were assayed for glucagon using a radioimmunoassay (Harris et al., 1979) and for epinephrine and norepinephrine levels using a single isotope radioenzymatic assay (Evans et al., 1978).

Statistical Analysis

All data are reported as mean ± standard error of the mean (SEM). The hormonal response to hypoglycemia is reported as the peak value achieved during the hypoglycemia period with the basal value subtracted. The hypoglycemia unaware group was compared to the control group with two-sample, two-sided Student's *t*-test, and after adjusting for plasma glucose with analysis of covariance. All analyses were carried out with SAS v8.2 (SAS Institute Inc., Cary, NC).

RESULTS

In total, 14 participants with type 1 diabetes and hypoglycemia unawareness verified by clinical history and Cox questionnaire and 27 healthy participants were studied. The two groups were similar with respect to body mass index and gender (Table I), but the hypoglycemia unaware group was on average 10 years older and there was little overlap in ages ($P = 0.0065$). No association appeared between age and brain glucose concentration (Spearman correlation = 0.18, $P = 0.26$), however, among all subjects studied. The secretory response of

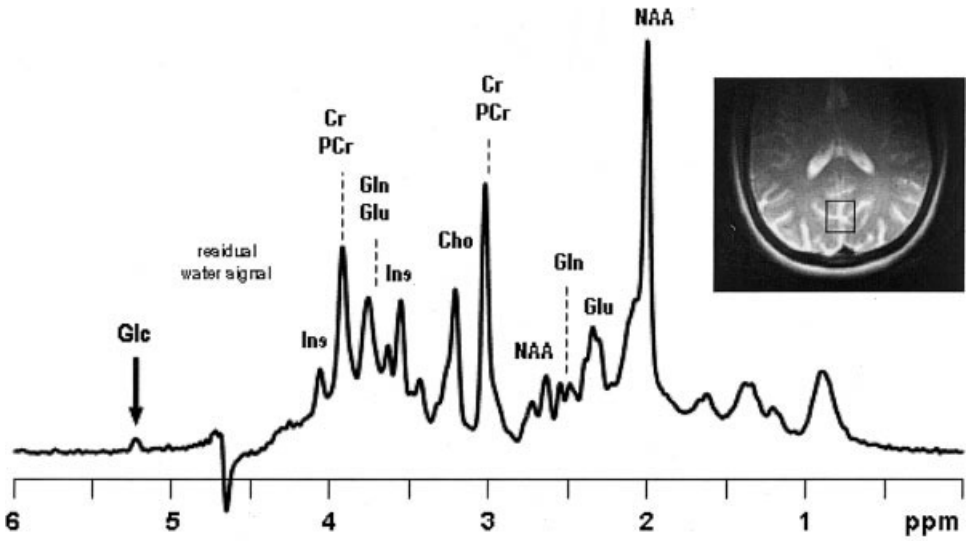

Fig. 2. ^{1}H MR spectra of a human brain during glucose infusion. ^{1}H MR spectroscopy at 4 Tesla was used to acquire a spectrum of a 16-ml volume of occipital cortex from a patient with diabetes when plasma glucose was clamped at 16.7 mmol/l. The peak at 5.23 ppm was used to quantify the brain glucose concentration. Glc, glucose; Cr, creatine; Ins, inositol; Cho, choline; Glu, glutamate; NAA, *N*-acetyl-aspartate.

TABLE I. Subject Characteristics*

Subject group	Gender (M/F)	BMI (kg/m^2)	Hemoglobin A1c (%)
Hypoglycemia unaware	11/3	26.7 ± 1.0	6.9 ± 0.2
Controls	17/10	26.7 ± 1.0	NA
P	0.32	0.98	NA

*M, male; F, female; BMI, body mass index.

glucagon, epinephrine, and norepinephrine during hypoglycemia was significantly lower in the subjects with type 1 diabetes and hypoglycemia unawareness as compared to that in controls, although not all of the subjects with hypoglycemia unawareness studied in the magnet protocol agreed to undergo the hypoglycemic clamp study (Table II). This blunted counterregulatory response was apparent despite the lower plasma glucose levels achieved in the hypoglycemia unaware group during the hypoglycemia clamp.

During the magnet experiment, the concentrations of plasma glucose in the two groups were maintained using the glucose/insulin clamp technique (Table III). Serum ketone measurements were negative in all subjects during the course of the experiment. Brain glucose concentrations were 17 ± 6% higher in the hypoglycemia unaware group than in the control group ($P = 0.016$). The brain glucose concentration in the hypoglycemia unaware group remained significantly higher than that in controls after adjusting for the difference in plasma glucose during the clamp (5.46 ± 0.3 vs. 4.8 ± 0.2; $P = 0.0367$). The brain-to-plasma glucose ratio was also higher in the hypoglycemia unaware group (0.33 ± 0.02 vs. 0.29 ± 0.01; $P = 0.0334$).

DISCUSSION

We found that patients with type 1 diabetes and hypoglycemia unawareness had higher brain glucose concentrations than healthy volunteers did when studied under the same hyperglycemic conditions. Because steady-state brain glucose concentration reflects the balance between glucose entering the brain across the blood brain barrier and glucose leaving the brain via metabolism, our findings support the hypothesis that recurrent hypoglycemia may increase cerebral glucose transport or decrease cerebral glucose metabolism. These observations provide evidence that the brain may compensate for chronic changes in glycemia so as to ensure a constant supply of its preferred energy substrate.

In evaluation of the difference observed, several factors must be taken into consideration. First, the standard error of the mean between the brain glucose concentrations measured in the hypoglycemia unaware group is greater than that the control group. Although all subjects were validated to have hypoglycemia unawareness and those who agreed to participate were shown to have a blunting of counterregulatory hormones in response to hypoglycemia, differences in the frequency of hypoglycemia and duration of hypoglycemia among subjects may account for the variability. Careful evaluation of the data failed to provide sufficient justification to omit any of the reported data points. We found no correlation between number of hypoglycemia episodes reported during the week before the experiment and brain glucose concentration (data not shown). We interpret these data to demonstrate that subjects with type 1 diabetes and hypoglycemia unawareness have a brain glucose concentration that is higher than that in normal controls studied under the same metabolic conditions. Although the difference may be numerically small, the clinical importance of the difference may be significant. Under normal conditions, the plasma glucose concentration is approximately four to five times higher than is the brain glucose concentration. During hypoglycemia (plasma glucose = 2.5 mmol/l), a 10% increase in brain glucose concentration (0.55 vs. 0.50 μmol/g wet weight) may be enough to have a clinical impact on the threshold for neuroglycopenic symptoms.

TABLE II. Hormonal Response to Hypoglycemia

Group	*n*	Plasma glucose during 30-min hypoglycemia (mmol/l)	Glucagon (pg/ml)	Epinephrine (pg/ml)	Norepinephrine (pg/ml)
Hypoglycemia unaware	6	2.4 ± 0.1	14 ± 4	288 ± 72	137 ± 26
Control	9	2.8 ± 0.1	85 ± 12	713 ± 116	270 ± 32
P	—	0.003	0.001	0.017	0.012

TABLE III. Results Obtained During Magnet Study Under Condition of Hyperglycemia*

Group	Serum insulin (pmol/l)	Plasma glucose (mmol/l)	GM [glucose] (μmol/g wet wt)
Hypoglycemia unaware	198 ± 24	16.7 ± 0.2	5.5 ± 0.3
Controls	138 ± 6	16.4 ± 0.1	4.7 ± 0.1
P	0.01	0.10	0.016

*GM [glucose], gray matter glucose concentration.

In our study, the plasma glucose concentrations in the patient group were on average slightly higher than those the control group, although the difference was not statistically significant. With the linear relationship that exists between plasma and brain glucose concentrations (Gruetter et al., 1998a; Choi et al., 2001; de Graaf et al., 2001; Seaquist et al., 2001), it is possible that this difference in plasma glucose concentration may have lead to an artifactual increase in brain glucose concentration in patients with diabetes. After adjusting for this difference statistically, however, the hypoglycemia unaware group still experienced significantly higher brain glucose concentration in gray matter.

Subjects with diabetes also had a significantly higher insulin concentration during the clamp study than did the controls although levels remained within the physiologic range. The increased plasma insulin concentration in the patient group may have been the result of subcutaneous depots of long acting insulin or a decreased insulin clearance. We have shown previously under the same experimental conditions that insulin is without a significant effect on the kinetics of cerebral glucose transport and metabolism in normal controls and that pharmacologic concentrations of plasma insulin do not change brain glucose concentrations (Seaquist et al., 2001). It is therefore unlikely that the difference in plasma insulin concentrations between the two groups can account for the difference in brain glucose concentration. PET studies have also shown that brain glucose metabolism is not sensitive to insulin concentration with the physiologic range (Cranston et al., 1998; Hasselbach et al., 1999).

The effects of recurrent hypoglycemia and hypoglycemia unawareness on brain glucose metabolism and the development of hypoglycemia-associated autonomic failure have been the subject of past investigation. Although upregulation of GLUT1 (Kumagai et al., 1995) and increased transporter concentration on the luminal surface of the blood–brain barrier (Simpson et al., 1999) have been demonstrated in animals with chronic hypoglycemia, these types of studies are impossible to replicate in otherwise healthy human beings. One alternative approach has been to measure brain glucose uptake as a surrogate for measures of glucose transport and metabolism. Using this methodology, Boyle et al. (1995) found that subjects with type 1 diabetes in good control had no change in brain glucose uptake in the face of systemic hypoglycemia, whereas subjects with type 1 diabetes in less optimal control and normal controls had a decrease in brain glucose uptake under the same condition of hypoglycemia. This evidence supports the hypothesis of a physiologic mechanism by which the brain protects itself from neuroglycopenia by preserving uptake. Other investigators have used PET imaging to measure the rate of blood-to-brain glucose transport and found no difference in the transport rate in healthy subjects after euglycemia compared to that in healthy subjects studied after a period of hypoglycemia (Segel et al., 2001). Similarly, McCrimmon et al. (2003), using microdialysis to measure the glucose concentration in the extracellular fluid of the inferior colliculus in rats, found that a prior exposure to recurrent hypoglycemia did not lead to an increase in glucose concentration, inferring that hypoglycemia did not cause an increase in blood–brain barrier glucose transport. Both of these studies were powered to detect a difference of greater than 20% and consequently were not able to detect a difference as small as the 16% noted in our study.

In summary, we found that patients with type 1 diabetes and hypoglycemia unawareness had an increased brain glucose concentration compared to that in healthy controls under similar levels of glycemia. This observation supports the hypothesis that recurrent hypoglycemia may alter cerebral glucose uptake or metabolism to preserve brain function in the context of systemic hypoglycemia. The exact mechanism by which the brain glucose concentration is increased in response to recurrent hypoglycemia and hypoglycemia unawareness remains unknown. Future investigation will be required to define clearly how this compensatory response occurs.

ACKNOWLEDGMENTS

We thank Dr. N. Tran and the superb nursing staff of the General Clinical Research Center at the University of Minnesota. This work was funded by grants from the National Institutes of Health (RO1-NS35192 to E.R.S.,

MO1 RR00400, and P41 RR08079 and the Juvenile Diabetes Research Foundation (13-2002-445 to A.B.C.).

REFERENCES

Adriany G, Gruetter R. 1997. A half-volume coil for efficient proton decoupling in humans at 4 tesla. J Magn Reson 125:178–184.

Amiel S, Sherwin R, Simonson D. 1988. Effect of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. Diabetes 37:901–907.

Boado R, Pardridge W. 1993. Glucose deprivation causes posttranscriptional enhancement of brain capillary endothelial glucose transporter gene expression via GLUT1 mRNA stabilization. J Neurochem 60:2290–2296.

Boyle P, Kempers S, O'Connor AM, Nagy R. 1995. Brain glucose uptake and unawareness of hypoglycemia in patients with insulin-dependent diabetes mellitus. N Engl J Med 333:1726–1731.

Boyle P, Nagy R, O'Connor A, Kempers S, Yeo R, Qualls C. 1994. Adaption in brain glucose uptake following recurrent hypoglycemia. Proc Natl Acad Sci USA 91:9352–9356.

Choi I, Lee S, Kim S, Gruetter R. 2001. In vivo measurements of brain glucose transport using the reversible Michaelis-Menten model and simultaneous measurements of cerebral blood flow changes during hypoglycemia. J Cereb Blood Flow Metab 21:653–663.

Choi I, Lei H, Gruetter R. 2002. Effect of deep pentobarbital anesthesia on neurotransmitter metabolism in vivo: on the correlation of total glucose consumption with glutamatergic action. J Cereb Blood Flow Metab 22:1343–1351.

Clarke W, Cox D, Gonder-Frederick L, Julian D, Schlundt D, Polonsky W. 1995. Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms. Diabetes Care 18:517–522.

Cranston I, Marsden P, Matyka K, Evans M, Lomas J, Sonksen P, Maisey M, Amiel SA. 1998. Regional differences in cerebral blood flow and glucose utilization in diabetic man: the effect of insulin. J Cereb Blood Flow Metab 18:130–140.

Cryer P. 1994. Hypoglycemia: the limiting factor in the management of IDDM. Diabetes 43:1378–1388.

Dagogo-Jack S, Craft S, Cryer P. 1993. Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. J Clin Invest 91:819–828.

DCCT Research Group. 1987. Feasibility of centralized measurements of glycated hemoglobin in the Diabetes Control and Complications Trial: a multicenter study. Clin Chem 33:2267–2271.

DCCT Research Group. 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:978–986.

de Graaf R, Pan J, Telang F, Lee J, Brown P, Novotny E, Hertherington H, Rothman D. 2001. Differentiation of glucose transport in human brain gray and white matter. J Cereb Blood Flow Metab 21:483–492.

Evans M, Halter J, Porte D. 1978. Comparison of double and single isotope enzymatic derivative methods of measurement of catecholamines in human plasma. Clin Chem 24:567–570.

Gruetter R. 1993. Automatic, localized in vivo adjustment of all first- and second-order shim coils. Magn Reson Med 29:804–811.

Gruetter R, Garwood M, Ugurbil K, Seaquist E. 1996. Observation of resolved glucose signals in ^{1}H NMR spectra of the human brain at 4 Tesla. Magn Reson Med 36:1–6.

Gruetter R, Tkac I. 2000. Field mapping without reference scan using asymmetric echo-planar techniques. Magn Reson Med 43:319–323.

Gruetter R, Ugurbil K, Seaquist E. 1998a. Steady-state cerebral glucose concentration and transport in the human brain. J Neurochem 70:397–408.

Gruetter R, Weisdorf SA, Rajanayagan V, Terpstra M, Merkle H, Truwit CL, Garwood M, Nyberg SL, Ugurbil K. 1998b. Resolution improvements in in vivo ^{1}H-NMR spectra with increased magnetic field strength. J Magn Reson 135:260–264.

Harris V, Faloona G, Unger R. 1979. In: Jaffe B, Behrman H, editors. Methods of hormone radioimmunoassay (2nd ed). New York: Academic Press, p 643.

Hasselbach S, Knudsen G, Videbaek C, Pinborg L, Schmidt J, Holm S, Paulson O. 1999. No effect of insulin on glucose blood–brain barrier transport and cerebral metabolism in humans. Diabetes 48:1915–1921.

Koranyi L, Bourey R, James D, Mueckler M, Fiedorek F, Permutt A. 1991. Glucose transporter gene expression in rat brain: pretranslational changes associated with chronic insulin-induced hypoglycemia, fasting and diabetes. Mol Cell Neurosci 2:244–252.

Kumagai A, Kang Y-S, Boado R, Pardridge W. 1995. Upregulation of blood–brain barrier GLUT-1 glucose transporter protein and mRNA in experimental chronic hypoglycemia. Diabetes 44:1399–1404.

Lee JH, Garwood M, Menon R, Adriany G, Andersen P, Truwit CL, Ugurbil K. 1995. High contrast and fast three-dimensional imaging at high fields. Magn Reson Med 34:308–312.

McCall A, Millington W, Wurtman R. 1982. Metabolic fuel and amino acid transport into the brain in experimental diabetes mellitus. Proc Natl Acad Sci USA 79:5406–5410.

McCrimmon R, Jacob R, Fan X, McNay E, Sherwin R. 2003. Effects of recurrent antecedent hypoglycemia and chronic hyperglycaemia on brainstem extra-cellular glucose concentrations during acute hypoglycaemia in conscious diabetic BB rats. Diabetologia 46:1658–1661.

Pardridge W. 1983. Brain metabolism: a perspective from the blood–brain barrier. Physiol Rev 63:1481–1535.

Pardridge W, Boado R, Farrell C. 1990. Brain-type glucose transporter (GLUT-1) is selectively localized to the blood–brain barrier: studies with quantitative Western blotting and in situ hybridization. J Biol Chem 265:18035–18040.

Petroff O, Spencer D, Alger J, Prichard J. 1989. High-field proton magnetic resonance spectroscopy of human cerebrum obtained during surgery for epilepsy. Neurology 39:1197–1201.

Rothman D, Petroff O, Behar K, Mattson R. 1993. Localized ^{1}H NMR measurements of GABA levels in human brain in vivo. Proc Natl Acad Sci USA 90:5662–5666.

Seaquist E. 1997. Comparison of arterialized venous sampling from the hand and foot in the assessment of in vivo glucose metabolism. Metabolism 46:1364–1366.

Seaquist E, Damberg G, Tkac I, Gruetter R. 2001. The effect of insulin on in vivo cerebral glucose concentrations and rate of glucose transport/metabolism in humans. Diabetes 50:2203–2209.

Segel S, Fanelli C, Dence C, Markham J, Videen T, Paramore D, Powers W, Cryer P. 2001. Blood-to-brain glucose transport, cerebral glucose metabolism, and cerebral blood flow are not increased after hypoglycemia. Diabetes 50:1911–1917.

Simpson IA, Appel NM, Hokari M, Oki J, Holman GD, Maher F, Koehler-Stec EM, Vannucci SJ, Smith QR. 1999. Blood–brain barrier glucose transporter: effects of hypo- and hyperglycemia revisited. J Neurochem 72:238–247.

Terpstra M, Ugurbil K, Gruetter R. 2002. Direct in vivo measurement of human cerebral GABA concentration using MEGA-editing at 7 Tesla. Magn Reson Med 47:1009–1012.

Tkac I, Starcuk Z, Choi I, Gruetter R. 1999. In vivo ^{1}H NMR spectroscopy of rat brain at 1 ms echo time. Magn Reson Med 41:649–656.

Yen S, Siler T, DeVane G. 1974. Effect of somatostatin in patients with acromegaly: suppression of growth hormone, prolactin, insulin and glucose levels. N Engl J Med 290:935–938.