Original Article

Increased Brain Monocarboxylic Acid Transport and Utilization in Type 1 Diabetes

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We hypothesized that increased capacity for brain utilization of nonglucose substrates (monocarboxylic acids [MCAs]) by upregulation of the MCA transporters may contribute metabolic substrates during hypoglycemia. To test this hypothesis, we assessed brain acetate metabolism in five well-controlled type 1 diabetic subjects and six nondiabetic control subjects using ^{13}C magnetic resonance spectroscopy during infusions of [2-^{13}C]acetate during hypoglycemia (~55 mg/dl). Acetate is transported into the brain through MCA transporters that are also used for lactate and ketones. Brain acetate concentrations were over twofold higher in the subjects with diabetes than the control subjects (P = 0.01). The fraction of oxidative metabolism from acetate (P = 0.015) and the rate of MCA transport (P = 0.01) were also approximately twofold higher in the diabetic subjects. We conclude that during hypoglycemia MCA transport in the brain was increased by appoximately twofold in patients with well-controlled type 1 diabetes, as reflected by higher brain acetate concentrations and rates of acetate oxidation. This upregulation would potentially allow a similar twofold increase in the transport of other MCAs, including lactate, during insulin-induced hypoglycemia. These data are consistent with the hypothesis that upregulation of MCA transport may contribute to the maintenance of brain energetics during hypoglycemia in patients with type 1 diabetes. *Diabetes* 55: 929–934, 2006

Under normal, nonfasting conditions, oxidation of glucose is the main source of energy for brain function (1). In intensively treated subjects with type 1 diabetes, there is often a loss of both the counterregulatory response and the mild cognitive symptoms before severe cognitive dysfunction (2,3). Both of these adaptations are believed to significantly contribute to hypoglycemic unawareness, which increases the risk of severe hypoglycemia, which may result in seizure or coma (4). The loss of cognitive symptoms is believed to be, at least in part, secondary to adaptations that allow brain energy metabolism to be maintained during moderate hypoglycemia (5). Several studies have found that intensively treated patients with type 1 diabetes have cortical metabolic adaptations during hypoglycemia. Positron emission tomography (PET) and arterio-venous difference studies have found that in contrast to control subjects who show a 20–30% decrease in glucose uptake during moderate hypoglycemia (~2.8 mmol/l), subjects with intensively treated type 1 diabetes show minimal reduction in glucose uptake (5–8).

Several hypotheses have been proposed to explain this metabolic adaption, particularly increased brain glucose transport, based on studies in rat models that showed an increase in glucose transporter activity in rats exposed to prolonged hypoglycemia (rev. in 9), but this concept is controversial. Studies of humans with diabetes have generally not been able to establish a change in transport parameters in poorly controlled and well-controlled patients. The study of Grill et al. (7) measured unidirectional glucose transport and found it to be the same in diabetic and control subjects in both normoglycemia and hypoglycemia. Another PET study (10) that used 3-O-methyl-D-glucose found no change in glucose transport parameters measured between type 1 diabetic and control subjects at normoglycemia and hyperglycemia. Similarly, a recent PET study (11) found no upregulation of glucose transport or uptake after exposure to antecedent hypoglycemia.

As early as 1990, Grill et al. (7) proposed that this adaptation may largely be due to increased utilization of alternate substrates to glucose, based on the finding that under euglycemic conditions glucose uptake was lower in the subjects with type 1 diabetes. Although glucose is the major substrate supporting metabolism in the central nervous system, under certain conditions, such as starvation, the brain can adapt to use alternative substrates such as ketones and lactate to meet its energy requirements (12–14). This adaptation, which is believed to involve an upregulation of monocarboxylic acid (MCA) transporters at the blood-brain barrier and possibly glial and neuronal membranes, allows normal cortical function at concentrations of glucose that under fed conditions would lead to hypoglycemic symptoms (~3.5 mmol/l).

Acetate is a MCA that has been shown to be transported into the brain by the same MCA transporters used for lactate and ketones. In the brain it is initially metabolized in the glial tricarboxylic acid (TCA) cycle (15,16). We and

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MCA, monocarboxylic acid; MRS, magnetic resonance spectroscopy; PET, positron emission tomography; TCA, tricarboxylic acid.

others have previously shown that acetate labeled with the stable isotope ^{13}C is an effective and relatively low-cost tracer for ^{13}C magnetic resonance spectroscopy (MRS) studies in humans (17). Analysis of ^{13}C labeling curves of glutamate, glutamine, and acetate during an infusion of [2-^{13}C]acetate allows determination of acetate transport and metabolism as well as relative rates of the glial and neuronal TCA cycle and glutamate/glutamine cycling to be calculated (18). In the present study, we used ^{13}C MRS in combination with infusion of [2-^{13}C] acetate to test the hypothesis that upregulation of transport and utilization of MCAs (acetate, acetoacetate, β-hydroxybutyrate, and lactate) in the brains of subjects with type 1 diabetes, who have previously experienced hypoglycemia associated with insulin use, might provide some substrate for energy generation as an alternative to glucose for brain metabolism. To address this hypothesis, we used ^{13}C MRS to measure the transport and utilization of [2-^{13}C]acetate during hypoglycemia at a level of ~3.1 mmol/l plasma glucose in the brains of two groups of subjects. One group consisted of subjects who had moderate- to well-controlled type 1 diabetes. The second group was made of nondiabetic control subjects matched for age and BMI.

RESEARCH DESIGN AND METHODS

Five young (aged 29 ± 4 years, BMI 25.1 ± 1.0 kg/m^2, three men and two women) healthy moderate- to well-controlled volunteers with type 1 diabetes and six healthy control subjects matched for age (aged 28 ± 2 years, five men and one woman) and BMI (24.8 ± 1.7 kg/m^2) were recruited for this study. They were all lean nonsmokers and taking no medications, except for insulin in the case of the subjects with diabetes. All subjects underwent a complete medical history and physical examination along with blood tests to verify normal blood and platelet counts, electrolytes, aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, creatinine, cholesterol, and triglycerides. The subjects with type 1 diabetes were all moderately well controlled, as reflected by a mean HbA_{1c} of 7.0 ± 0.5%, and all had a history of multiple episodes of hypoglycemia unawareness within the preceding 2 months, based on a history of low blood glucoses done by finger-stick home glucose monitoring associated with unawareness of symptoms by the patient on multiple occasions. Written consent was obtained from each subject after the purpose, nature, and potential complications of the studies had been explained. The protocol was approved by the Yale University Human Investigation Committee.

Measurement of metabolites and hormones. Plasma glucose and lactate concentrations were measured every 5–10 min with the use of a YSI 2700 STAT Analyzer (Yellow Springs Instruments). Samples for hormones were taken every 15 min. Plasma concentrations of insulin and glucagon were measured with the use of double-antibody radioimmunoassay kits (Linco, St. Charles, MO). Plasma epinephrine and norepinephrine were measured with a three-step procedure that consisted of adsorption onto alumina (pH 8.6), elution with dilute acid, and analysis by high-pressure chromatography. Fractional enrichments and plasma acetate concentrations were measured from blood samples collected at 10-min intervals and analyzed on a Hewlett-Packard 5890 gas chromatograph (HP-1 capillary column; 12 × 0.2 × 0.33-mm film thickness; Hewlett Packard, Palo Alto, CA) interfaced to a Hewlett-Packard 5971A mass selective detector operating in the electron impact ionization mode.

Hypoglycemic clamp studies. All subjects were admitted to the Yale–New Haven Hospital General Clinical Research Center the evening before the study. Dinner was served at 6:00 P.M., and the subjects were fasted overnight until the end of the study the following day. The subjects with diabetes were given an overnight intravenous insulin infusion (Novolin; Novo Nordisk, Bagsværd, Denmark) in order to achieve and maintain euglycemia overnight. At 6:00 A.M. the next morning, an intravenous catheter was inserted into each antecubital area for blood collection and for infusions, while the intravenous insulin was continued. The catheters were kept patent with 0.9% saline. Basal blood samples were collected for the determination of plasma glucose, lactate, insulin, catecholamines, and glucagon concentrations. Blood for determination of these substrates and hormones were collected every 30 min throughout the study. At ~8:00 A.M., the subjects were brought to the magnetic resonance center, the insulin drip was discontinued for 1–3 min, and the subjects were placed in the supine position in the MRS scanner. The subjects were positioned so the back of the head was over the center of the ^{13}C MRS receiver coil. After the MRS acquisition parameters were adjusted and baseline MRS spectra were acquired, a primed-continuous infusion of insulin was initiated, and after 10 min priming the rate was kept constant at 40 mU/(m^2 − min). Plasma glucose concentrations were measured every 5 min, and plasma glucose was allowed to decrease to 3.1 mmol/l and kept constant at that level with a variable infusion of 20% dextrose. When the plasma glucose concentrations reached 3.1 mmol/l, a primed-continuous infusion of [2-^{13}C]acetate (Isotec, Miamisburg, OH) was started and continued for 90–120 min at a rate of 68 μmol · kg body wt^{-1} · min^{-1} (3 mg · kg^{-1} · min^{-1}). Magnetic resonance spectra were acquired continuously throughout the study, and blood samples were drawn at intervals of 5–10 min for the determination of plasma substrate and hormone concentrations and for determination of the enrichment of plasma [2-^{13}C]acetate.

MRS acquisition. MRS data were acquired on a 2.1 T whole-body (1-m bore) magnet connected to a modified Bruker AVANCE spectrometer (Bruker Instruments, Billerica, MA). Subjects lay supine in the magnet, with the head lying on top of a radio-frequency magnetic resonance probe consisting of one 8.5-cm-diameter ^{13}C circular coil and two ^1H quadrature coils for ^1H acquisition and decoupling. After tuning, acquisition of scout images, shimming with the FASTERMAP procedure (19), and calibration of the decoupling power, ^{13}C MRS spectra were acquired for 10 min before and during the [2-^{13}C] acetate infusion. Magnetic resonance spectra were acquired using an ISIS localized adiabatic $^{13}C[^1H]$ polarization transfer sequence optimized for detection of glutamate and glutamine in the C4 position (20). The spectroscopic voxel was located in the occipital-parietal lobe, with dimensions of 6 × 4 × 6 cm^3.

MRS spectral analysis. The free induction decays were added in running averages of 15 min (i.e., three 5-min blocks). The spectral data were prepared for analysis with −1-Hz/4-Hz lorentzian-to-gaussian conversion and 16-fold zero-filling followed by Fourier transformation. An LC model approach (21,22) was used to fit the peak areas of glutamate and glutamine C4, taking into consideration the lower detection efficiency of glutamine C4 as measured in solution. Briefly, the LC-Model approach used model spectra of C4-labeled ^{13}C glutamate and ^{13}C-labeled glutamine, which were fitted to the data, using a spline baseline fit and treating any lipid contamination as three Gaussian peaks. The peak widths and heights of the three Gaussian lines and the model peaks of glutamate and glutamine were adjusted to obtain least-squares fits to the spectral data. The ratio of acetate utilization to the astrocytic TCA cycle rate (CMR_{Ac}/V_{tcaA}) was calculated based on the steady-state enrichments of glutamate and glutamine C4 using a series of differential equations describing a two-compartmental model as previously described (18).

Metabolic modeling analysis. Previous studies have demonstrated that acetate is initially metabolized in the brain almost exclusively by the glial TCA cycle (18 and references therein). Therefore, at isotopic steady state, assuming that the rate of isotopic equilibration of mitochondrial α-ketoglutarate and glutamate is rapid, the following is the isotope balance equation for the carbon four of glial glutamate (Glu_G):

$$100 \times (dGlu_{A4}^{*}/dt) = APE_{Ac}CMR_{Ac} + APE_{Glu_{N4}}V_{cycle} \quad (1)$$

$$-APE_{Glu_{A4}}(V_{gln} + V_{tcaANet}) = 0,$$

where CMR_{Ac} is the cerebral metabolic rate for acetate, V_{cycle} is the rate of glutamate-glutamine neurotransmitter cycling, $V_{tcaANet}$ is the rate of the astrocytic TCA cycle from α-ketoglutarate to succinate and beyond (the V_{tcaA} from citrate to α-ketoglutarate, minus V_{efflux}), and APE_{Ac}, APE_{GluN4}, and APE_{GluA4} are the fractional isotopic enrichments of acetate, neuronal glutamate carbon 4, and astrocytic glutamate carbon 4, respectively. Because glial glutamate is the precursor of glutamine, their steady-state enrichments are equal, so $APE_{Gln4} = APE_{GluA4}$. Also, V_{gln} is the sum of V_{cycle} and the rate of glutamine efflux (V_{efflux}). By mass balance for glial glutamate, $V_{tcaANet} + V_{efflux} = V_{pdhA} + CMR_{Ac}$. Finally, because neuronal glutamate constitutes most of the total glutamate pool, $APE_{Glu4} \sim APE_{GluN4,}$ and substitution into Eq. 1 yields the following:

$$\frac{CMR_{Ac}}{V_{tcaA}} = (APE_{Gln4}/APE_{Ac}) - \frac{V_{cycle}}{V_{tcaA}}\frac{APE_{Glu_4} - APE_{Gln4}}{APE_{Ac}} \quad (2)$$

Statistical analysis. To detect statistically significant differences between control subjects and type 1 diabetic subjects, we used unpaired Student's t tests for independent samples, with a two-tailed P value of <0.05 considered to indicate statistical significance. All data are presented as means ± SD in the text unless otherwise noted.

RESULTS

Type 1 diabetic subjects manifested a defective counter-regulatory response to hypoglycemia, as reflected by a

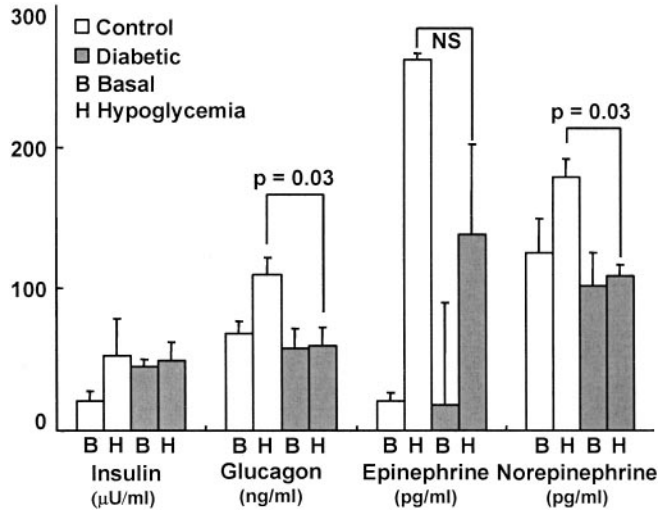

FIG. 1. Plasma concentrations of insulin and counterregulatory hormones before and during the hypoglycemic clamp (means ± SE). The glucagon and norepinephrine counterregulatory response to hypoglycemia was significantly blunted in the diabetic subjects.

blunted increase in plasma glucagon and norepinephrine concentrations compared with the control subjects (Fig. 1) and a higher glucose infusion rate (diabetic: 12.2 ± 2.2 mmol · kg^{-1} · min^{-1}, control: 6.6 ± 1.1 mmol · kg^{-1} · min^{-1}) during the hypoglycemic clamp.

Basal concentrations of glucose (control: 93 ± 11 mg/dl, diabetic: 109 ± 39 mg/dl) and lactate (control: 0.95 ± 0.15 mmol/l, diabetic: 1.03 ± 0.38 mmol/l) were similar in both control and diabetic subjects before the start of the hypoglycemic clamp. Following initiation of the hypoglycemic clamp, plasma glucose concentration decreased to a mean value of 56 ± 4 mg/dl in the control subjects and 54 ± 2 mg/dl in the diabetic subjects within 20–25 min and was clamped at the level for the duration of the study in both groups. Plasma lactate concentrations tended to increase in the control subjects (1.65 ± 0.25 mmol/l) during hypoglycemia but remained constant in the type 1 diabetic subjects (1.08 ± 0.17 mmol/l). Once the subjects reached a plasma glucose concentration of 55 mg/dl, an infusion of [2-^{13}C]acetate was begun and plasma acetate concentrations reached a steady-state within 15 min in both control (0.79 ± 0.12 mmol/l) and diabetic (1.04 ± 0.04 mmol/l) subjects. Following the start of the [2-^{13}C]acetate infusion, brain C4 glutamine and C4 glutamate became rapidly labeled in both groups (Fig. 2). However, the rate and amount of ^{13}C label incorporated into these positions was approximately two- to threefold greater in the type 1 diabetic subjects compared with the control subjects (Fig. 3), which was much more than could be accounted for by the slightly higher plasma concentrations of acetate in the diabetic subjects. ^{13}C incorporation into the C3 carbons of glutamine and glutamate from [2-^{13}C]acetate occurs during the second turn of the TCA cycle, and labeling in these positions appeared similar in both groups (Fig. 3).

During hypoglycemia acetate utilization, as a fraction of astrocytic TCA cycle rate, was estimated with Eq. 1 to be twofold greater in the diabetic (0.34 ± 0.09) compared with the control (0.17 ± 0.06; $P = 0.015$) subjects (Fig. 4). The steady-state concentration of brain acetate in the diabetic subjects (0.07 ± 0.02 mmol/kg) was 2.2 times greater than in the control subjects (0.03 ± 0.01 mmol/kg) ($P = 0.01$, Fig. 5). Note that based upon estimates of the brain blood volume of ~3% (23), plasma acetate could account for almost all of the acetate measured in the control subjects and ~40% of that measured in the subjects with type 1 diabetes.

From these data, it can be estimated that brain MCA transport activity was increased by approximately twofold as follows: For the consumption of acetate and the bidirectional transport mediated by a passive, saturable carrier that follows Michaelis-Menten kinetics, the equation is

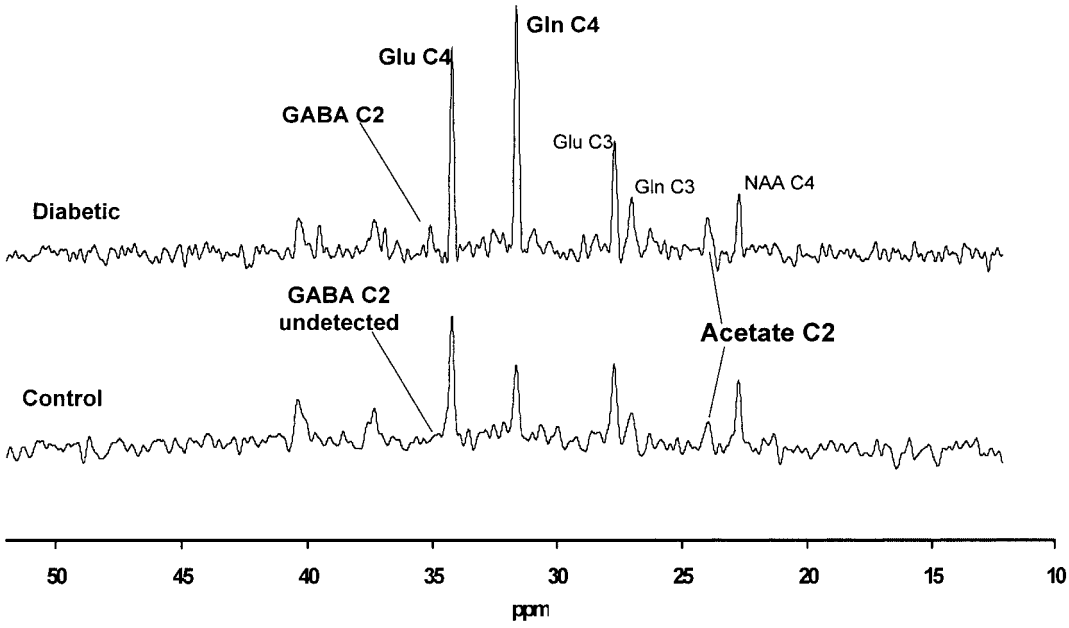

FIG. 2. Representative brain ^{13}C spectra from subjects with and without type 1 diabetes averaged over the final 45 min of the studies during hypoglycemia, while [2-^{13}C]acetate was infused. The subject with type 1 diabetes (*top spectrum*) had significantly greater labeling in the C4 position of glutamate (Glu C4) and glutamine (Gln C4) than the control subject (*bottom spectrum*). The acetate C2 signal (Acetate C2) was also greater in the diabetic than the control subjects. Other resonances labeled in the figure are from *N*-acetyl aspartate (NAA C4), GABA (GABA C2), and the C3 positions of glutamate (Glu C4) and glutamine (Gln C3).

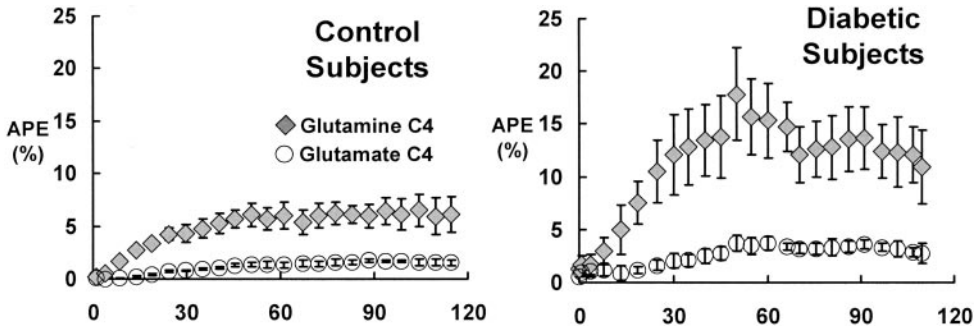

FIG. 3. Average time courses of labeling of the C4 carbons of glutamate and glutamine for the two groups of subjects. The subjects with type 1 diabetes had between two- to threefold higher labeling of both molecules, indicating greater utilization of [2-^{13}C]acetate in the individuals with type 1 diabetes.

$$\frac{d[\text{Acetate}_{in}]}{dt} = \frac{V_{max_T}[\text{Acetate}_{out}]}{K_{m_T} + [\text{Acetate}_{out}]} - \frac{V_{max_T}[\text{Acetate}_{in}]}{K_{m_T} V_d + [\text{Acetate}_{in}]} - \text{CMR}_{Ac} \quad (3)$$

which at steady state can be rearranged to

$$\frac{V_{max_T}}{V_{tca_A}} = \frac{\frac{\text{CMR}_{Ac}}{V_{tca_A}} (K_{m_T} V_d + [\text{Acetate}_{in}]) (K_{m_T} + [\text{Acetate}_{out}])}{K_{m_T}([\text{Acetate}_{out}]V_d - [\text{Acetate}_{in}])} \quad (4)$$

where [Acetate$_{in}$] and [Acetate$_{out}$] are the concentrations of acetate inside and outside of the brain. K_{mT} and V_{maxT} are the Michaelis-Menten constants of half-saturation maximum rate. K_{mT} for acetate was assumed to be similar to that of acetoacetate and lactate, or ~0.7 (24,25) (This assumption does not affect the relative rates of V_{maxT}/V_{tcaA} between the diabetic and control subjects.) V_d is the brain water space of 0.77 mg/dl (26). In the equations, it is assumed that the brain and plasma pH are the same in both groups, due to the protonated species of acetate being transported. Applying Eq. 4 to estimate V_{maxT} for each subject, using each individual's values of [Acetate$_{in}$], [Acetate$_{out}$], and CMR$_{Ac}$/V_{tcaA}, as measured by ^{13}C MRS, V_{maxT}/V_{tcaA} was found to be 1.9-fold higher in the diabetic (0.69 ± 0.17) compared with the control (0.36 ± 0.08, P = 0.01) subjects. Note that the actual upregulation in the subjects with type 1 diabetes may be somewhat higher because the majority of the brain acetate measured in the control subjects was most likely in the blood, so that acetate transport in these subjects was effectively unidirectional.

DISCUSSION

In this study, we examined the hypothesis that upregulation of MCA transport and utilization by the brain during hypoglycemia may contribute significantly to brain energetics by providing the brain with alternative energy sources other than glucose. In support of this hypothesis, we found that brain acetate concentration and utilization were both increased by more than twofold in the diabetic compared with the control subjects. This could be attributed to an approximately twofold inducement of brain acetate transport activity. Acetate is transported into the brain by MCA transporters, which are also responsible for transporting lactate and ketone bodies (i.e., β-hydroxybutyrate, acetoacetate, acetone, acetate), so an increase in the transport capacity of acetate is expected to indicate an increase in the transport capacity of the other MCAs. All of these substrates are capable of supporting brain metabolism during hypoglycemia.

Consistent with this hypothesis is the finding that brain lactate uptake was increased in well-controlled insulin-treated streptozotocin-induced diabetic dogs during hypoglycemia compared with nondiabetic control dogs (27). Furthermore, exogenously supplied lactate and β-hydroxybutyrate have been associated with lower blood glucose concentrations before symptoms of hypoglycemia appear (28), and lactate infusions have been reported to improve cognitive function during hypoglycemia and to do so more effectively in diabetic patients (29).

To increase net uptake of lactate, the blood-brain gradient must increase, either by increasing plasma lactate or

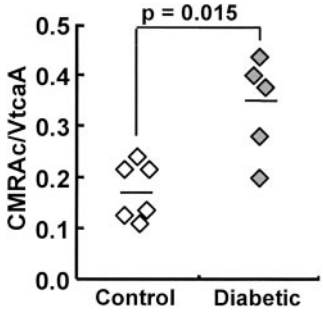

FIG. 4. Brain acetate utilization during hypoglycemia. As a fraction of the astrocytic TCA cycle rate, the subjects with type 1 diabetes (◆) had a twofold greater increase in brain acetate utilization than the control subjects (◇).

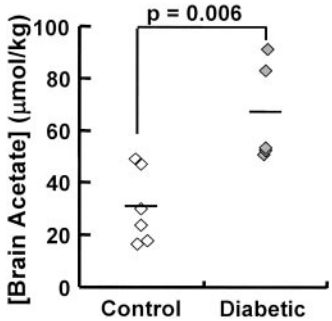

FIG. 5. Brain acetate concentration during hypoglycemia. The subjects with type 1 diabetes (◆) had over twice the amount of brain acetate as the control subjects (◇).

decreasing brain lactate. In the present measurements, plasma lactate levels remained relatively constant, but during hypoglycemia, brain lactate levels must fall as the glucose substrate for lactate production is depleted. Finally, recent studies have demonstrated that infusion of lactate into the hypothalamus of rats abolishes the glucose counterregulatory response to hypoglycemia (30). By increased MCA transport, it is possible that any of several of the MCAs discussed could provide additional substrate for energy production during hypoglycemia. Several studies (27,28,31,32) of insulin-induced hypoglycemia make lactate in particular a prime candidate.

An estimate of the amount of energy that must be supplied by MCA to sustain brain energy metabolism during moderate hypoglycemia may be calculated from the studies of Grill et al. (7), who found a reduction in brain glucose uptake of ~15–20% of that seen in control subjects during moderate hypoglycemia of 50 mg/dl, assessed by PET (8). Similar results have been reported using arteriovenous difference methods by Boyle (5). Under insulin-induced hypoglycemic conditions lactate is normally the MCA with the highest plasma concentration (>1 mmol/l in both groups). Under normal conditions unidirectional transport of lactate into the brain is ~15% of the rate of glucose uptake (33,34). A twofold increase in lactate transport due to the upregulation of MCA transport activity, measured in the present study, is therefore sufficient to account for the reduction in availability of glucose as a fuel during moderate hypoglycemia. Consistent with this possibility, Pan et al. (35) have shown that a 3-day fast, which reduced the brain's glucose requirements by 20–30%, increased brain lactate concentration by more than twofold despite no increase in plasma lactate concentrations.

An alternative possibility for explaining the metabolic resistance to hypoglycemia observed in type 1 diabetes is an upregulation of glucose transport. Although upregulation has been shown in rodent hypoglycemia models (9), there has been no conclusive evidence in humans. Grill et al. (7) measured unidirectional glucose transport and found it to be the same in diabetic and control subjects under both normoglycemia and hypoglycemia. Another PET study that used 3-O-methyl-D-glucose found no change in glucose transport parameters measured between type 1 diabetic and control subjects at normoglycemia and hyperglycemia (10). Similarly a recent PET study found no upregulation of glucose transport or uptake after exposure to antecedent hypoglycemia (11) sufficient to induce blunted counterregulation. Recently a study using ^{1}H MRS has reported a 10% increase in plasma glucose concentrations during hyperglycemia in subjects with well-controlled type 1 diabetic with reported episodes of hypoglycemia (36). It was proposed that this increase was due to an equivalent increase in brain glucose transport activity. However, this difference can be equally well explained due to a downregulation in glucose consumption as a consequence of increased MCA consumption. This explanation would be consistent with reports that during euglycemia, brain glucose uptake is reduced in well-controlled type 1 diabetes (7). Future studies examining the metabolism of other MCAs such as lactate by ^{13}C MRS should be able to distinguish these possibilities conclusively.

In summary, this is the first study to demonstrate increased brain MCA transport activity in patients with well-controlled type 1 diabetes. The results are consistent with the hypothesis that upregulation of monocarboxylic acid transporters in the brain of well-controlled diabetic patients may play a major role in maintaining cerebral energy metabolism during hypoglycemia in patients with type 1 diabetes.

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