PRELIMINARY REPORT

Brain Glucose Levels Are Elevated in Chronically Hyperglycemic Diabetic Rats: No Evidence for Protective Adaptation by the Blood Brain Barrier

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Recent evidence suggests that brain function may be impaired by prolonged elevations of blood glucose, such as those that occur in poorly controlled diabetes. However, little is known about the effects of such hyperglycemia on brain metabolic substrate levels. Using microdialysis in awake, freely moving rats, we directly measured brain extracellular fluid (ECF) glucose, lactate, and β-hydroxybutyrate (βOHB) levels in the inferior colliculus in chronically hyperglycemic BB/wor diabetic rats and in control (Sprague-Dawley) rats during euglycemia and acute hyperglycemia. The ECF:plasma glucose ratio (0.27 to 0.34) was remarkably similar in animals from all 3 groups, resulting in proportional elevations of brain ECF glucose in the hyperglycemic groups. Moreover, brain ECF levels of lactate and β-OHB were increased in diabetic (DM) rats as compared with controls. Our results suggest that no significant protective adaptation of the blood brain barrier (BBB) transfer of glucose occurs in chronic hyperglycemia. Hence, brain tissue may be chronically exposed to markedly elevated levels of glucose and other metabolic fuels during poorly controlled diabetes, and therefore it may be subject to the same long-term adverse effects of hyperglycemia seen in peripheral tissues.

IN POORLY CONTROLLED diabetes, chronically elevated blood glucose levels increase the risk of long-term diabetic complications, including those of the kidney, retina, and peripheral nervous system.[1] The central nervous system has not, traditionally, been thought of as a target of damage associated with diabetic hyperglycemia. However, recent evidence suggests that the brain may indeed be damaged by such conditions. A report utilizing data from approximately 10,000 women showed a significant association between the presence of diabetes (and duration of disease) and impaired cognitive performance. [2] Unlike peripheral organs, the brain is protected from direct metabolic contact with the blood by the blood brain barrier (BBB). Little is known about the effects of diabetes and/or hyperglycemia on the metabolic composition of the brain's extracellular fluid (ECF). Therefore, we measured, using ECF microdialysis, the effects of hyperglycemia on the equilibration of glucose and other substrates across the BBB. To distinguish any effects of acute hyperglycemia from the prolonged hyperglycemia that is characteristic of type 1 diabetes, we used both chronically hyperglycemic BB/wor rats, an animal model of poorly controlled diabetes, and control rats made acutely hyperglycemic by dextrose infusion.

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MATERIALS AND METHODS

One week before study, male Sprague Dawley (351 ± 13g) and aged matched BB/wor diabetic rats (299 ±12g, duration of diabetes = 59 ± 9 days) were placed under ketamine:xylazine anesthesia while chronic vascular catheters were inserted and microdialysis guide cannulae positioned bilaterally in the inferior colliculus region of the auditory brainstem using standard stereotaxic techniques.[3] Subsequently, BB/wor rats received daily injections of protamine zinc insulin at a dose sufficient to maintain body weight, but insufficient to prevent chronic hyperglycemia. All animal care procedures were reviewed by the Yale Animal Care and Use Committee and followed the Principles of Laboratory Animal Care.

On the morning of study, the vascular catheters were flushed and microdialysis probes (CMA-12, 3mm 20 kD cutoff membrane, CMA/Microdialysis, N Chelmsford, MA) were inserted through the guide cannulae. Artificial ECF was perfused through both probes at 0.3 μL/minute. After a 2-hour probe equilibration interval, animals were studied in the fed state without restraint for 2 additional hours using one of the following protocols. Nondiabetic Sprague-Dawley rats received either a saline infusion (euglycemia, N = 21) or a variable rate 10% dextrose infusion (N = 6) to acutely raise and maintain plasma glucose at hyperglycemic levels. Chronically hyperglycemic BB diabetic rats received a variable dextrose infusion to maintain them at their morning glucose level (≃ 22 mmol/L, N = 18).

In each protocol, microdialysis aliquots were collected during the final 60 minutes of the 2-hour study period when plasma glucose had reached steady state. Dialysate glucose and lactate were measured with an automated CMA600 analyzer. Plasma glucose was measured with a Beckman Glucose II analyzer (Fullerton, CA). Plasma and dialysate β-hydroxybutyrate (β-OHB) were measured by a fluoroenzymatic technique.

The microdialysis technique can be influenced by a variety of factors, such as perfusate flow rate and composition, such that dialysis levels are generally less than true ECF concentrations. As a result, to estimate true ECF substrate levels and facilitate comparisons between laboratories, each method must be calibrated.[4] Correction for probe recovery for glucose and lactate on the basis of in vitro recovery measurements for each individual probe was validated in preliminary experiments. In vitro probe recovery for glucose and lactate averaged 37% ± 4% and 64% ± 5%. Individual probe recoveries for β-OHB

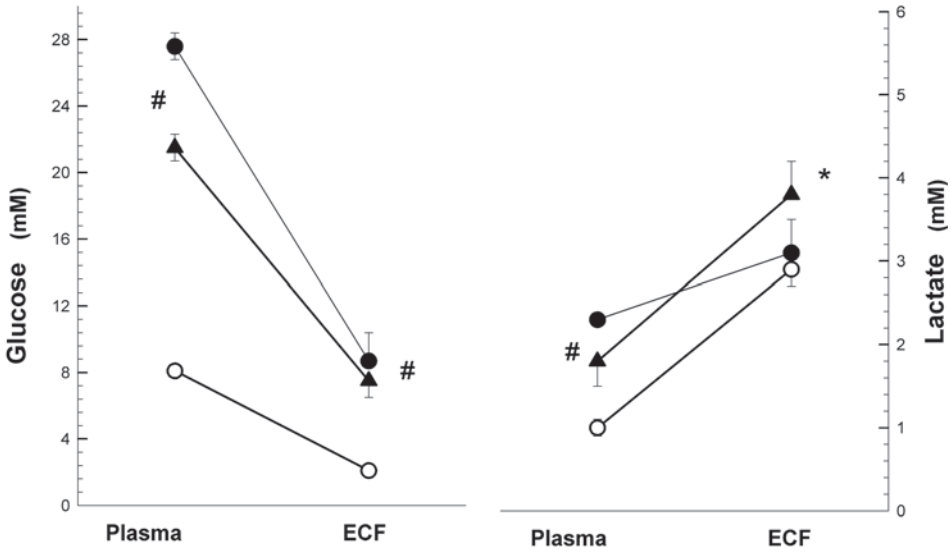

Fig 1. A comparison of glucose and lactate concentrations in plasma and brain ECF. Three groups were studied; euglycemic (○) and acutely hyperglycemic (●) nondiabetic rats and chronically hyperglycemic diabetic rats (▲). Data are expressed as mean ± SEM. #P < .01 for hyper and DM *v* euglycemic controls. *P < .05 for DM v euglycemic controls.

were not performed because in vitro data using similar probes showed that β-OHB and lactate recovery were indistinguishable. Therefore, individual probe data for lactate recovery were used to estimate absolute ECF β-OHB levels. Data were analyzed using analysis of variance (ANOVA) and are presented as mean ± SE.

RESULTS AND DISCUSSION

As shown in Fig 1, plasma glucose was significantly elevated during acute hyperglycemia and diabetes compared with euglycemic controls. Although brain ECF glucose was lower than plasma in all groups, ECF levels were markedly elevated during hyperglycemia (8.7 ± 1.7) and diabetes (7.5 ± 1.0) compared with controls (2.1 ± 0.2 mmol/L). Importantly, the ratio of ECF glucose to plasma glucose was remarkably constant across the groups (no significant differences; 0.27 ± 0.3 controls, 0.32 ± 0.06 hyperglycemic controls, 0.34 ± 0.04 DM rats). Previous studies of BBB transport[5] suggest the endothelial glucose transporter may be regulated in response to changes in blood glucose level, with the number of endothelial transporters decreasing with hyperglycemia. However, our results suggest that any such regulation induced by chronic hyperglycemia has minimal impact, if any, on transport of glucose from the blood to the brain's ECF. Hence, brain tissue in chronically hyperglycemic BB/wor rats, and possibly poorly controlled diabetic patients, will be exposed to prolonged elevation of glucose.

As expected, plasma β-OHB was markedly elevated in the DM rats compared with both Sprague-Dawley groups (5.3 ± 1.1 mmol/L *v* 0.2 ± 0.1). β-OHB was easily detected in brain ECF from diabetic rats (1.8 ± 0.7mmol/L), but not from either nondiabetic group. The brain ECF-to-plasma ratio of 0.34 obtained for β-OHB was remarkably similar to that observed for glucose; consistent with entry of ketones across the BBB via facilitated diffusion under conditions of elevated plasma ketone levels. Several in vivo studies have suggested that the brain can use ketones, at least in part, as an alternative nonglucose fuel.[6]

Like glucose, plasma lactate was elevated in both acute (2.3 ± 0.02) and chronic hyperglycemic (1.8 ± 0.3 mmol/L) groups compared with the level seen in euglycemic controls (1.0 ± 0.1, $P < .01$ *v* both hyperglycemic groups). Unlike glucose and β-OHB, however, ECF lactate was significantly higher than the level found in plasma, consistent with data supporting brain lactate production.[7] In addition, brain ECF lactate concentration in the diabetic rats (3.8 ± 0.4), but not the acutely hyperglycemic nondiabetic group (3.1 ± 0.4) was significantly higher than in euglycemic controls (2.9 ± 0.2 mmol/L), implying that the elevation may be a consequence of prolonged, rather than acute, hyperglycemia. It is uncertain, however, whether this adaptation to prolonged hyperglycemia occurs as a consequence of decreased neuronal usage, increased neuronal or astrocytic glycolysis, or altered BBB transport.

In summary, brain tissue in poorly controlled diabetes is not shielded from the potential adverse cellular effects of chronic hyperglycemia and may be exposed to excessive levels of alternative metabolic fuels (lactate and ketones), as well as glucose.

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