Brain oxygen utilization is unchanged by hypoglycemia in normal humans: lactate, alanine, and leucine uptake are not sufficient to offset energy deficit

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Submitted 7 February 2005; accepted in final form 30 August 2005

Lubow, Jeffrey M., Ivan G. Piñón, Angelo Avogaro, Claudio Cobelli, David M. Treeson, Katherine A. Mandeville, Gianna Toffolo, and Patrick J. Boyle. Brain oxygen utilization is unchanged by hypoglycemia in normal humans: lactate, alanine, and leucine uptake are not sufficient to offset energy deficit. Am J Physiol Endocrinol Metab 290: E149–E153, 2006. First published September 6, 2005; doi:10.1152/ajpendo.00049.2005.—During hypoglycemia, substrates other than glucose have been suggested to serve as alternate neural fuels. We evaluated brain uptake of endogenously produced substrates other than glucose have been suggested to serve as alternate neural fuels. We evaluated brain uptake of endogenously produced lactate, alanine, and leucine at euglycemia and during insulin-induced hypoglycemia in 17 normal subjects. Cross-brain arteriovenous differences for adjacent neurons (10).

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compensate for acutely reduced BGU. We find that none of these potential alternate fuels can account for more than 25% of the loss of ATP induced by reducing the systemic glucose concentration to 3.0 mmol/l in normal humans.

MATERIALS AND METHODS

Subjects. Seventeen normal human volunteers (11 men, 6 women) participated in the study. Eleven were non-Hispanic whites, two were Hispanic, two were Native American, one was African American, and one was Asian American. The nature, purpose, and possible risks of the study were carefully explained to the subjects before they agreed to participate. The Human Research and Review Committee of the University of New Mexico (UNM) School of Medicine approved the protocols and informed consents. All subjects were recruited from flyers posted on campus at UNM, and all reported that they had no chronic or acute medical problems and were taking no regular medications. All underwent a history and physical examination ~1–2 wk before the study, at which time a screening blood was collected for a chemistry panel, complete blood count, liver function tests, cholesterol level, and a quantitative β-hCG for women.

Experimental protocol. All subjects were admitted to the UNM General Clinical Research Center the evening before the study. Subjects fasted for 8 h before the start of the study and continued fasting until the end. At 0600, an 18-gauge peripheral intravenous line was placed in or near the right antecubital fossa to subsequently deliver insulin and dextrose. At 0630, a 21-gauge radial arterial line was inserted under local lidocaine anesthesia. This was followed by passage of a retrograde internal jugular catheter to the level of the right jugular bulb. Our group and others have previously used roentgenography to demonstrate proper placement of the cannula at the jugular bulb by this technique (6, 28).

Measurement of cerebral blood flow. For measurement of cerebral blood flow (CBF), subjects rested in a 1-ft³ plastic tent with a high flow of medical air flowing through it to prevent humidity from building up and to allow rapid introduction of the tracer gas. A mixture of 9% N₂O and 21% oxygen was then delivered for 25 min while simultaneous arterial and venous blood samples for N₂O were collected. We have noted that the shorter time frames suggested by Kety and Schmidt (18) may fall short of allowing equilibrium to be established in some subjects. Samples for arterial and venous N₂O concentrations were collected at 30-s intervals for 6 min and then every 2 min until 25 min.

Brain glucose uptake (BGU) is the product of the CBF and the arteriovenous difference (A-V)ₐₚ for glucose across the brain. Utilization of other brain substrates were similarly determined. As with other substrates, brain oxygen uptake (CMRO₂) was calculated by multiplying the CBF by the (A-V)ₐₚ for oxygen content across the brain. CBF was estimated using the method of Fick, utilizing the equation developed by Kety and Schmidt (18): CBF = 1000VₐS/(1000[A-V]dr). The arterial concentration rises to equilibrium values more rapidly than the venous compartment, and the area between the two curves is inversely related to the CBF. Vₐ in the equation is the venous equilibrium concentration, and S is the partition coefficient for N₂O between the blood and the brain at equilibrium. S is known to be 1.0 in humans (17). A Statistical Analysis Software (SAS) program (32) was written to perform multiple iterations to fit the best sigmoidal curve to data set as previously described (7). The program forces convergence at the final equilibrium point. The coefficients derived from the iterative process were used to calculate theoretical y-axis values at 0.1-min intervals, and the area between these finite increments was calculated so that the area under the curves was determined by 10.220.33.2 on June 25, 2017 http://ajpendo.physiology.org/ Downloaded from
of \(-1.8 \pm 0.6\) to a net uptake of \(2.5 \pm 1.2 \mu\text{mol} \cdot 100 \text{g}^{-1} \cdot \text{min}^{-1}\) \((P < 0.001)\), whereas mean brain leucine uptake (BLeuU) decreased from a net uptake of \(5.5 \pm 1.0\) to \(1.9 \pm 0.4 \mu\text{mol} \cdot 100 \text{g}^{-1} \cdot \text{min}^{-1}\) \((P < 0.003)\), and mean brain alanine uptake (BAU) tended to decrease from \(2.1 \pm 1.8\) to \(-1.9 \pm 1.6 \mu\text{mol} \cdot 100 \text{g}^{-1} \cdot \text{min}^{-1}\), although this was not statistically significant \((P < 0.08)\). Arterial lactate concentration rose from \(0.85 \pm 0.09\) to \(1.90 \pm 0.15 \text{mmol/l}\) \((P < 0.001)\), whereas the arterial concentrations of both alanine and leucine decreased from \(0.32 \pm 0.02\) to \(0.26 \pm 0.02 \text{mmol/l}\) \((P < 0.009)\) and from \(0.11 \pm 0.01\) to \(0.03 \pm 0.003 \text{mmol/l}\) \((P < 0.001)\), respectively.

**DISCUSSION**

In agreement with our prior investigations (6), in the face of a 27% decline in the uptake of glucose by brain, we observed
no significant change in CMRO₂ during hypoglycemia in normal humans, although CMRO₂ did display a slight downward trend from 164 ± 17 to 151 ± 13 μmol·100 g⁻¹·min⁻¹, euglycemia to hypoglycemia, (P = 0.32). Our values of 164 and 151 μmol·100 g⁻¹·min⁻¹ fall within the range of normal values seen in the literature (7, 13, 15, 24). Two basic possibilities exist to explain these facts: 1) fuels other than glucose were being oxidized as provision of glucose from the circulation fell, or 2) there was a change in the rate of glucose oxidation (either aerobic or anaerobic). The brain relies on anaerobic glycolysis, as ~15% of brain glucose is converted to lactate and does not enter the TCA cycle (30). The potential exists that more pyruvate could be diverted to the more energy-yielding (and oxygen-utilizing) TCA cycle as hypoglycemia develops. Fox et al. (13) demonstrated a dramatic increase in BGU assessed by 18-fluorodeoxyglucose positron emission tomography with visual stimulation. In the face of this dramatic increase in glucose utilization, a minimal increase in CMRO₂ occurred, indicating an increase in anaerobic glycolysis. As such, one might envision a decrease in BGU during hypoglycemia being associated with a fall in anaerobic glycolysis and a rise in TCA oxidation. We did not measure changes in rates of glucose oxidation (either aerobic or anaerobic); therefore, we cannot be certain whether or not a change in the percentage of each possible metabolic process for glucose utilization may have occurred. The brain uptake of at least one of the substrates we measured did increase during hypoglycemia.

The rate of BLuU increased whereas BLeuU fell, and BAU remained essentially unchanged. Quantitatively, the reduction in BGU of 9.7 μmol·100 g⁻¹·min⁻¹ observed in the current investigation translates into a fall in ATP generation of 291 μmol·100 g⁻¹·min⁻¹. On the other hand, uptake of lactate by the brain increased from a net loss of ~1.8 to a net gain of 2.5 μmol/100 g/min. Such a change translates into an absolute increase in ATP of 74 μmol·100 g⁻¹·min⁻¹, or 25% of the energy deficit incurred from diminished BGU. Although the pharmacological elevation of lactate concentration has been shown to decrease physiological symptoms of hypoglycemia and attenuate the counterregulatory hormonal response, a normal, acute elevation of endogenous lactate production during hypoglycemia (and consequent increase in BLU) is inadequate to satisfy the loss in brain energy metabolism from reduced glucose uptake.

One limitation to our investigation is that the arteriovenous differences of any of the substrates were small and we could have missed a lesser change. Others, like Avogaro et al. (3), have generated large arterial concentrations, and consequently larger arteriovenous differences, which lead to dramatic increments in BLU. This led them to postulate that lactate could potentially replace glucose as the primary brain energy substrate during hypoglycemia. Such findings stimulated the first investigations in humans that demonstrated that infusion of exogenous lactate or ketones could partially attenuate the counterregulatory response associated with insulin-induced hypoglycemia and help prevent cerebral dysfunction in type 1 diabetes, presumably through increased lactate utilization for fuel (12, 22, 34).

Lactate is generated locally by glial cells and presented to neurons, serving as a primary neuronal fuel (10). Brain extracellular fluid lactate concentrations rise during hypoglycemia (1), suggesting that there is either increased lactate production or decreased lactate clearance. Given that neuronal tissue appears to utilize lactate, it would seem illogical to conclude that lactate uptake would decrease in the face of an energy deficit. Therefore, brain lactate utilization (or uptake from the circulation as we have shown) should be expected to increase during hypoglycemia.

The possibility remains that exogenous insulin may have influenced our results. We did not measure blood or brain insulin levels, and it is therefore difficult to speculate regarding any effect resulting from exogenous insulin administration. Certainly the brain has large numbers of insulin receptors, but their function is probably not related to glucose transport or utilization, although controversy still exists on this issue (5, 31).

One might speculate that brain glycogen could serve as a transient source of fuel during acute hypoglycemia and, if oxidized, would lead to stable oxygen utilization in the face of reduced brain glucose uptake from the circulation. Recent NMR spectroscopy data have suggested that the amount of brain glycogen available for mobilization for energy during hypoglycemia may be larger than previously believed (9), and this may contribute to the observed increase in brain extracellular lactate such as that seen by Abi-Saab et al. (1). Choi et al. (9) demonstrated depletion of brain glycogen during insulin-induced hypoglycemia in rats, concluding that brain glycogen may play a significant role in brain metabolism during acute hypoglycemia. Furthermore, they demonstrated a supercompensation of brain glycogen generation following a single episode of hypoglycemia, opening the door to the possibility that clinical recurrent hypoglycemia in patients with diabetes may result in greater and greater ability to compensate for subsequent hypoglycemia. Thus, glycogen breakdown and combustion could also explain why we failed to observe a fall in CMRO₂. No human investigations that demonstrate utilization of brain glycogen exist.

We documented a significant decrease in BLeuU during hypoglycemia in normal humans. In addition to finding a decrease in BLeuU, we found a significant decrease in arterial leucine levels during hypoglycemia vs. euglycemia, a finding also noted by Battezzati et al. (4). Thus diminished substrate delivery from decreased peripheral production seems the likely explanation for the reduced rate of BLeuU. Although we observed no significant change in BAU during hypoglycemia, there was a tendency for BAU to decrease (P < 0.08), whereas arterial plasma alanine significantly decreased (P < 0.009). Davis et al. (11) studied levels of numerous endogenous compounds in dogs during hypoglycemia. Their results were similar to ours, with arterial blood alanine decreasing and arterial blood lactate increasing significantly during both peripheral and head insulin infusions.

In conclusion, we find that none of the potential alternate fuels we evaluated could account for more than 25% of the loss of ATP induced by reducing the systemic glucose concentration to 3.0 mmol/l in normal humans. The applicability of our findings to the setting of humans with type 1 diabetes who experience recurrent bouts of hypoglycemia is limited. Improved uptake and utilization of alternate fuels in response to repeated episodes of neuroglycopenia would be metabolically advantageous; however, this hypothesis remains to be tested.
ACKNOWLEDGMENTS

We gratefully acknowledge the skills of the nursing staff of the General Clinical Research Center.

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GRANTS

This research was supported by National Institute of Neurological Disorders and Stroke Grants 1 R29 NS-29972-01A1 and K24 NS-02097-05, by dedicated Health Research Funds of the University of New Mexico, and by a grant from General Clinical Research Program, Division of Research Resources 5 M01 RR-00997-28.

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