Acute hypoglycemia induces increased brain lactate uptake and metabolism in rats.

H. M. De Feyer1, K. L. Behar2, R. A. de Graaf3, and D. L. Rothman4,5

1Diagnostic Radiology, Yale University, New Haven, CT, United States, 2Department of Psychiatry, Yale University, 3Diagnostic Radiology, Yale University, 4Department of Biomedical Engineering, Yale University

Introduction
Repetitive mild hypoglycemic events in patients with Type 1 diabetes lead to brain adaptations that blunt the warning signals associated with low glucose levels (hypoglycemia unawareness) (1). Increased capacity to oxidize alternative monocarboxylic acid (MCA) fuels, e.g., lactate and ketone bodies, associated with increased blood-to-brain transport of MCA's by monocarboxylate transporter 1 (MCT1), has been suggested as an adaptation contributing to hypoglycemia unawareness (2). Of the MCAs present in the blood during hypoglycemia, lactate is present at the highest concentration. Increased uptake and oxidation of blood-borne lactate could (partially) replace glucose, preserving brain energy metabolism and thereby contribute to hypoglycemia unawareness and failing counterregulatory response. To further investigate the role of blood lactate as an alternative fuel during hypoglycemia, we studied brain lactate transport and metabolism in healthy rats during both hyperinsulinemic euglycemia and acute hypoglycemia using In vivo 1H[13C] magnetic resonance spectroscopy (MRS) in combination with [3-13C]-lactate infusion.

Materials and methods
Long Evans rats were anesthetized with isoflurane, tracheotomized and ventilated using ~30% O2 and ~70% N2O. One femoral artery and two femoral veins were catheterized for blood sampling and blood pressure measurements and infusion of insulin, glucose and [3-13C]-lactate, respectively. A heating pad was used to maintain body temperature at 37°C. All in vivo NMR measurements were performed using a 9.4T horizontal bore magnet interfaced to a Varian spectrometer. A combined quadrature 1H and single loop 1H surface coil set-up was placed on top of the skull to acquire [1H-13C] spectra from a 180 μl voxel (6 × 5 × 6 mm3) positioned in the middle of the cortex. A Proton-Observed-Carbon-Edited (POCE) sequence was applied with a repetition time of 2.5 s and total echo time of 25 (17 + 8) ms (3). After scout imaging, voxel positioning and subsequent infusion, insulin infusion was started (0.02 μL/min/g body weight, 2.5 U/ml solution). When a steady state plasma glucose level of ~6 mM (euglycemia) or ~2.3 mM (hypoglycemia) was achieved by applying variable glucose infusion rates, a primed infusion of [3-13C]-lactate (0.157 μL/min/g body weight, 0.5 mM solution) and in vivo MRS acquisition were started. POCE difference spectra were fitted using an LC model approach with in-house built software. Plasma lactate concentration and enrichment were determined using a spin-echo sequence at a 500 MHz high resolution Bruker spectrometer. Data are presented as mean ± standard deviation.

Results
Mean plasma glucose levels were 5.8 ± 0.7 and 2.3 ± 0.3 mM in euglycemic and hypoglycemic rats, respectively (n=5). Steady state plasma lactate concentrations were 3.3 ± 0.5 mM during euglycemia and 3.6 ± 0.9 mM for hypoglycemia, whereas fractional enrichments of plasma lactate where 19.8 ± 1.25 and 1 ± 0.37 % in euglycemic and hypoglycemic rats, respectively. Figure 1a depicts the 13C signal of glutamate C4 (Glu4) expressed in arbitrary units, determined after scaling total C13C spectra to the creatine peak at 3.02 ppm. The 13C signal of Glu4 in hypoglycemic rats is ~ twice the level of euglycemic rats. Mean 13C fractional enrichment calculated for Glu4 from spectra acquired after 75 min of lactate infusion (example of spectra shown in Figure 1b) was 6.3 ± 0.7 % for euglycemic animals and 13.0 ± 1.8 % for hypoglycemic animals. To confirm the apparent fast change in brain lactate transport and metabolism two-stage glycemic clamps were performed within the same animals resulting in euglycemic levels followed by acute hypoglycemia (by reducing the glucose infusion rate), while keeping the [3-13C]-lactate infusion constant (n=3, see Figure 2). Glu4 13C fractional enrichments were 7.4 ± 0.7 % at average glucose concentration of 6.4 ± 0.2 mM (45 min) and increased to 11.9 ± 0.2 % when glucose was dropped to 2.3 ± 0.7 mM (120 min).

Discussion
Levels of 13C fractional enrichment of Glu4 during infusion of [3-13C]-lactate were increased following acutely induced hypoglycemia compared to rats under euglycemic conditions, suggesting a rapid upregulation of lactate transport activity upon lowering plasma glucose levels. Indeed, glycemic clamp studies in which the same rats were studied under both euglycemic and hypoglycemic conditions confirmed the rapid increase in lactate transport and/or oxidation, as Glu4 enrichment increased to a new steady state level when glucose levels were lowered. The higher level of Glu4 13C enrichment induced by hypoglycemia can be the result of increased brain metabolism of lactate, a higher transport capacity of lactate at the blood-brain barrier or a combination of both. A higher brain lactate transport capacity could indicate hypoglycemic induction of activated/translocated MCT1 in the endothelial cells of brain blood vessels. Further studies are underway to determine the contributions of transport and oxidation of lactate, which should provide new insights into the capacity of blood-borne lactate as fuel for brain energy metabolism. The ability of the brain to rapidly upregulate MCA transport and metabolism during hypoglycemia may be an important mechanism for adapting to hypoglycemia and could play an important role in the pathogenesis of hypoglycemia unawareness.

References

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