Increased brain lactate transport and metabolism during hypoglycemia in rats fed a ketogenic diet

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Introduction

Intensive insulin treatment to control blood glucose levels in individuals with type 1 or advanced type 2 diabetes places them at increased risk for iatrogenic hypoglycemia. Repetitive mild hypoglycemic events lead to brain adaptations (Hypoglycemia Associated Autonomic Failure, HAAF)(1) resulting in failing hormone counterregulatory response and lack of the warning symptoms (hypoglycemia unawareness) normally associated with decreasing glucose levels. Increased capacity to oxidize alternative monocarboxylic acid (MCA) fuels (e.g. lactate and ketone bodies) associated with increased blood-brain barrier MCA transport *via* the MCA transporter 1 (MCT1), has been suggested as adaptations induced by repetitive hypoglycemia (2). This hypothesis is based on findings in well-controlled type 1 diabetes patients who showed increased metabolism of acetate, a molecule also transported over the blood-brain barrier *via* MCT1 (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 (2).

To further investigate the role of blood lactate as an alternative fuel during hypoglycemia, we studied brain lactate transport and metabolism in rats maintained on a ketogenic diet, which is known to enhance MCT1 expression (3). *In vivo* ^{1}H-[^{13}C] magnetic resonance spectroscopy (MRS) was used in combination with [3-^{13}C]-lactate infusion. Preliminary *in vivo* results of ^{13}C labeling of glutamate during [3-^{13}C]-lactate infusion under hyperinsulinemic-hypoglycemic conditions are presented.

Materials and methods

Long Evans rats were fed normal chow (NC, n=3) or a ketogenic diet (KD, n=3) based on 91% fat and 9% protein (Harlan Teklad, # TD96355) for 15-16 days. Daily caloric intake was matched to guarantee similar body weights at the time of the [3-^{13}C]-lactate infusion study. After an overnight fast rats were anesthetized with isoflurane, tracheotomized and ventilated using ~30% O_2 and ~70% N_2O. One femoral artery and two femoral veins were catheterized for blood sampling and blood pressure measurements and infusion of insulin, glucose and [3-^{13}C]-lactate, respectively. A heating pad was used to maintain body temperature at 37°C, measured with a rectal probe. All *in vivo* NMR measurements were performed using a 9.4T horizontal bore magnet interfaced to a Varian spectrometer. A combined quadrature ^{13}C and single loop ^{1}H surface coil set-up was placed on top of the skull to acquire ^{1}H-[^{13}C] spectra from a 180 μL voxel ($6 \times 5 \times 6$ mm^3) positioned in the middle of the cortex. The Proton-Observed-Carbon-Edited (POCE) sequence was applied with a repetition time of 2.5 s and echo time of 25 ms (3). After scout imaging, voxel positioning and shimming, insulin infusion was started (0.02 μL/min/g body weight, 2.5 U/ml solution). When a steady state plasma glucose level of ~ 2.3 mM was achieved by applying variable glucose infusion rates, a primed infusion of [3-^{13}C]-lactate (0.157 μL/min/g body weight, 0.5 mM solution) and *in vivo* MRS acquisition were started. Ninety minutes after the onset of [3-^{13}C]-lactate infusion the animal was frozen *in situ* in liquid nitrogen during continuous ventilation and the brain stored at -80°C. POCE difference spectra were fitted using an LC model approach with in-house built software. Plasma lactate concentration and ^{13}C enrichment were determined using a POCE sequence at a 500 MHz high resolution Bruker spectrometer. Data are presented as mean ± standard deviation.

Results

Body weight of both groups of animals at time of study were comparable (NC: 228 ± 10; KD: 220 ± 17 g). Plasma glucose levels during the hypoglycemic clamps were 2.2 ± 0.4 and 2.3 ± 0.4 mM in NC and KD animals, respectively. Sixteen minutes after the start of the [3-^{13}C]-lactate infusion plasma lactate concentrations and enrichment were 1.7 ± 0.3 mM and 32.5 ± 3.6 % in NC animals; and 1.8 ± 0.4 mM and 39.1 ± 3.9% in KD rats. ^{13}C labeling of glucose was not detected. Figure 1a depicts the ^{13}C signal of glutamate C4 and lactate C3, expressed in arbitrary units, determined after scaling all original spectra to the creatine peak at 3.02 ppm. KD rats showed a robust increase in ^{13}C-labeled lactate and glutamate levels compared to control animals fed normal chow. Figure 1b shows POCE difference spectra averaged over the last 45 min of [3-^{13}C]-lactate infusion acquired from a KD and NC animal.

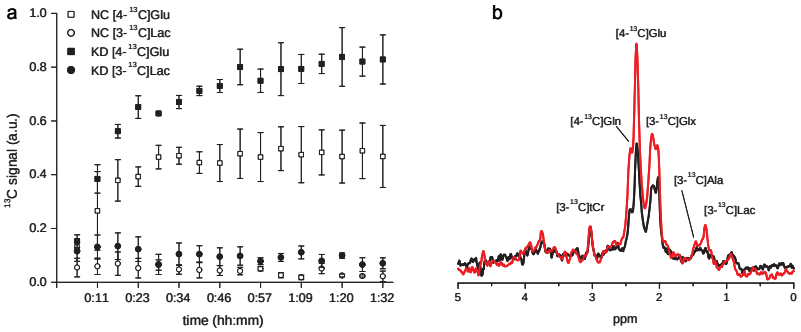

Figure 1 a) ^{13}C signal amplitude of brain glutamate (Glu) and lactate (Lac) of fitted POCE difference spectra during the infusion of [3-^{13}C]-lactate. **b)** POCE difference spectra averaged over the last 45 min of [3-^{13}C]-lactate infusion of a NC (black) and KD (red) rat. Peak annotations: tCr: total creatine; Glu: glutamate; Gln: glutamine; Glx: glutamate+glutamine; Ala: alanine; Lac: lactate. Original spectra were scaled at the 3.02 ppm tCr peak before calculation of the difference spectra.

Discussion

The ketogenic diet as used in the present study has been shown to induce upregulation of MCT1 expression at the blood-brain barrier (3)**.** KD rats displayed a large increase in brain glutamate C4 ^{13}C labeling compared to NC rats, despite similar blood lactate levels and ^{13}C enrichments, consistent with an increase in blood-to-brain lactate transport and/or metabolism in KD rats. We conclude that adaptations in response to the ketogenic diet enhance lactate transport and/or metabolism in rats during hypoglycemia. Whether such effects enhance support of neurons and/or astroglia during hypoglycemia or provides neuro-protection remains to be shown. Further studies are underway to disentangle 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. Such new insights have relevance both to diabetes and to conditions treated with ketogenic diets such as epilepsy (5).

References

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