Cerebral glucose uptake in humans at hypoglycemic plasma levels follows reversible Michaelis-Menten kinetics

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Introduction

Cerebral glucose levels in humans can be measured non-invasively by ^{13}C MRS to determine the kinetics of glucose transport into the brain. This transport can be described by a reversible Michaelis-Menten (MM) model [1], which is characterized by a half-maximal transport constant (K_t) and maximal transport rate (T_{max}) or maximal transport rate relative to CMR_{glc} (T_{max}/CMR_{glc}). Several studies investigated the validity of the MM kinetic model and have determined these kinetic parameters for the brain of healthy subjects [1,2], for white (WM) and gray matter (GM) separately [3,4] and for the effect of insulin [4]. However, all these studies focused on brain glucose content as a function of plasma glucose levels under euglycemic (plasma glucose ~5 mM) and hyperglycemic (plasma glucose up to 30 mM) conditions. The effects of hypoglycemia on cerebral glucose levels have been studied by proton MRS [5,6] in humans, but have not been quantified and kinetic parameters were not presented. In rats it was demonstrated that the MM kinetic properties hold for hypoglycemic plasma levels of < 5 mM glucose and that values of the kinetic parameters are comparable to what has been reported for humans [7]. Another study demonstrated that chronic hypoglycemia increases brain glucose and consistently increases T_{max}/CMR_{glc} [8]. *The aim of this study was to measure human cerebral glucose content **during** hypoglycemia using ^{13}C MRS, in order to calculate values of reversible MM kinetic parameters for glucose transport and to compare these with previously reported data on glucose transport into the brain.*

Methods

Eight healthy volunteers (4 male/4 female, 23.2±2.5 yrs old) were subjected to two hyperinsulinemic (60 $mU \cdot m^{-2} \cdot min^{-1}$) glucose clamps after an overnight fast [9]. They were clamped at euglycemia (~5mM) on one day and at hypoglycemia (~3mM) on another day, 4 weeks apart. The clamps were designed to provide stable and comparable plasma levels of [1-^{13}C]glucose. As such, a bolus of 6 g of 100% [1-^{13}C]glucose 20 % (w/w) solution was infused over 10 minutes followed by infusion of 40% and 50% enriched [1-^{13}C]glucose for the euglycemic and hypoglycaemic clamp, respectively, at a variable rate to maintain target plasma glucose levels. Arterial blood was sampled every 5 min to determine plasma glucose concentration and [1-^{13}C]glucose isotopic enrichment by high resolution ^{1}H NMR. For in vivo measurements a DEPT sequence was used in combination with ISIS localization and ^{1}H decoupling. ^{13}C-MRS acquisition (72 scans, TR=2s, duration=2.5 min) of a voxel of ~125 ml in the occipital brain was started 20 min before clamping to obtain 8 reference spectra, and continued throughout the entire clamp (± 2 h). All experiments were performed at 3T with an optimized volume coil for ^{1}H with a CP surface coil insert for ^{13}C [10].

Post-processing and quantification: MR spectra acquired during steady-state (~5.0 and ~3.0 mM, t=50-100 min) were averaged and then phased in the region 70 – 100 ppm (including glucose and myo-inositol signals) with jMRUI. The peaks of α- and β-glucose in the summed spectra were fitted with the AMARES algorithm. To quantify metabolite levels the natural abundance ^{13}C myo-inositol signals were assumed to represent 1.1% of 6 mM [11]. In addition, glucose signals were corrected for the pulse profile as measured in a phantom, the ^{13}C enrichment of plasma glucose and for 5% contamination by blood vessels.

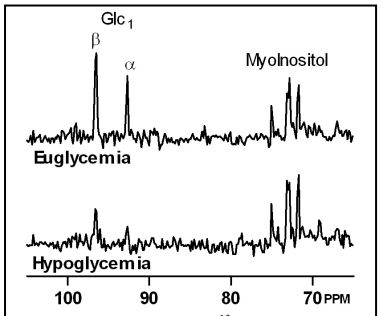

Figure 1: representative ^{13}C MR spectra of one volunteer under euglycemia (top) and hypoglycemia (bottom), scaled to myo-inositol signals.

Results

In ^{13}C MR spectra of the brain there was an obvious difference in glucose signal intensity between the euglycemic and hypoglycemic state with respect to the natural abundance myo-inositol signals (Fig.1). Steady-state brain glucose glucose levels averaged at 0.50±0.22 µmol/g at hypoglycemia (plasma glucose = 2.99±0.33 mM) and 1.26±0.50 µmol/g at euglycemia (plasma glucose = 5.05±0.29 mM). Individual steady-state brain glucose levels as a function of plasma glucose at hypo- and euglycemic clamp conditions are presented in figure 2A, together with the best fit of the data and a 95% confidence interval. By linear regression we derived from a reversible MM kinetic model values for the parameters: T_{max}/CMR_{glc} = 2.93 and K_t = 3.27 mM. In figures 2B and 2C, we plotted our data points together with the linear relations between plasma and brain glucose levels reported previously in humans at euglycemic and hyperglycemic conditions [1,4] and in rats under hypoglycemic conditions [7].

Discussion and conclusion

Since glucose is the primary fuel of the brain, it is important to characterize brain glucose values as a function of plasma glucose values especially at hypoglycemia. Previously, brain glucose levels and values for MM kinetic parameters were estimated for euglycemic and hyperglycemic plasma levels, with rather large standard deviations that made these values less reliable for hypoglycemic conditions [1,4]. In our study we demonstrated that under moderate hypoglycemic conditions signals for glucose in the brain were still detectable in all subjects. Furthermore, the estimated brain glucose levels are in line with extrapolation of brain glucose values at higher plasma glucose concentrations and within the error of MM parameters published for this data ([1]: T_{max}/CMR_{glc} = 2.3±0.2, K_t = 0.6±2.0 mM. [4]: WM: T_{max}/CMR_{glc} = 2.15±0.25, K_t = 1.96±2.45 mM, WM: T_{max}/CMR_{glc} = 2.24±0.23, K_t = -0.98±2.13 mM). Our data agrees even better with a rat study performed during hypoglycemia ([7]: T_{max}/CMR_{glc} = 2.7±0.13, K_t = 3.3±1.0 mM), which also show estimated brain glucose levels to approach zero at plasma glucose levels of ~2 mM. Whether the linear reversible MM kinetic relationship still holds at even lower plasma glucose values in humans is cannot be derived from our data. We conclude that we successfully measured brain glucose values at moderate hypoglycemia (~3 mM) in humans, and kinetic parameters confirm a reversible MM model for glucose transport at hypoglycemia.

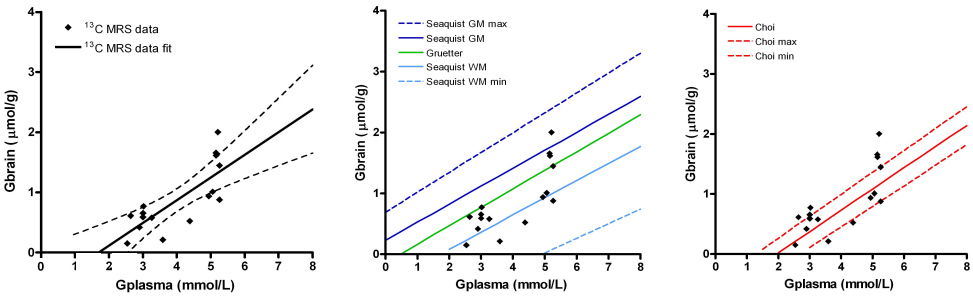

Figure 2: A) Brain glucose (Gbrain) values as a function of plasma glucose values (Gplasma) as measured with ^{13}C MRS (diamonds), corresponding best fit (solid line) and 95% confidence interval (dashed lines). B) ^{13}C MRS data (diamonds) and fits based on kinetic parameters published by Gruetter [1] (green solid line), and in the presence of insulin in GM (dark blue solid line) and WM (light blue solid line) as presented by Seaquist [4]. The dashed lines represents the maximum and minimum estimates based on average MM parameters ± 1 sd. C) ^{13}C MRS data (diamonds) together with best fit (solid red line) of data in rats [7], and average MM parameters ± 1 sd (dashed red lines).

References: 1. Gruetter et al., J Neurochem 1998; **2.** Duarte et al., Front Neuroenergetics 2009; **3.** De Graaf et al., J Cereb Blood Flow Metab 2001; **4.** Seaquist et al., Diabetes 2001; **5.** Bisschof et al., Eur J Clin Inv 2006; **6.** Heikkila et al., Diabetologia 2009; **7.** Choi et al., J Cereb Blood Flow Metab 2001; **8.** Lei et al., J Neurochem 2006; **9.** Van de Ven et al., J Neurosci Methods. 2010; **10.** Klomp et al., MRM, 2006 **11.** Ross B, et al., NMR in Biomed, 2003.

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