13C MRS to study human brain metabolism during HYPOglycemia is feasible

M. van der Graaf[1], B. E. de Galan[2], D. W. Klomp[1], C. J. Tack[2], A. Heerschap[1]

[1]Radiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, [2]Medicine, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands

Introduction

In type-1 diabetes, intensive insulin therapy resulting in recurrent hypoglycemic events may induce hypoglycemia unawareness, in which lower levels of glycemia are required to initiate counterregulatory hormone release and to experience hypoglycemia warning symptoms [1]. Despite possible roles for brain glycogen [2], blood flow and glucose transport [3], the underlying mechanism in the development of hypoglycemia unawareness still needs to be elucidated. So far, little is known about the effect of hypoglycemia on cerebral glucose metabolism vis-à-vis glucose delivery and general energy demand.

13C MRS during 13C-1-glucose infusion can be used to follow uptake and metabolism of 13C-labeled glucose. Generally, such experiments are performed under HYPERglycemic conditions to ensure sufficient signal-to-noise ratio of the 13C-labeled metabolite signals. The **aim** of this feasibility study was to optimize the conditions for 13C MRS of the human brain during a HYPOglycemic clamp to allow studies on human brain metabolism under hypoglycemic conditions.

Methods

Subjects: After an overnight fasting period, two healthy 23-yrs old female volunteers underwent a hyperinsulinemic hypoglycemic glucose clamp [4] with 13C-1-glucose infusion using extended tubing inside the bore of a 3T Siemens Trio MR system. Approval of the local ethics committee was obtained and the volunteers gave written informed consent.

Clamp conditions: At time t = -5 min. a continuous insulin infusion of 60 $mU \cdot m^{-2} \cdot min^{-1}$ was started, followed by a bolus of (A) 30 ml of 50% 13C-1-glucose 20% (w/w) solution or (B) 30 ml of 100% 13C-1-glucose 20 % (w/w) solution during 10 minutes at the initiation of the clamp (start at t = 0 min). During the remainder of the experiment 50% 13C-1-glucose 20% (w/w) solution was infused in both subjects. After the initial bolus used to enhance 13C enrichment in the blood, plasma glucose was allowed to fall to ~ 3.0 mM and maintained at that level for about 2 hrs. Arterial blood was sampled every 5 min. to determine plasma glucose levels and 13C-1-glucose isotopic enrichment (afterwards by high resolution 1H NMR).

13C-MRS experiments: 13C-MRS experiments were performed at 3T using an optimized volume coil for 1H with a CP surface coil insert for 13C [5]. We used a ISIS-DEPT sequence with proton decoupling (WALTZ-16) in which 1H ISIS localization was combined with signal enhancement by 1H-13C polarization transfer. An alpha pulse of 45 degrees in the DEPT sequence was used to allow simultaneous observation of CH, CH_2, and CH_3 13C MR signals. 13C MR spectra (72 scans, TR = 2 sec) from a voxel of ~ 125 ml covering occipital brain tissue were acquired during the entire clamp period with a time resolution of 2.5 min. For reason of visualization, 8 spectra were added and a 20-min spectrum obtained prior to infusion was subtracted to remove some small baseline distortions due to lipid signals.

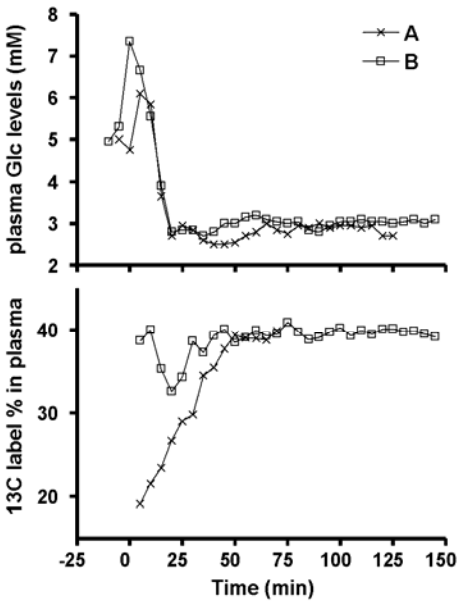

Figure 1

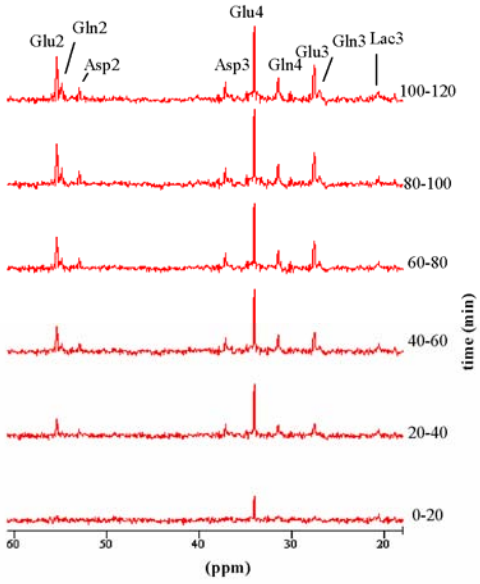

Figure 2

Results

Figure 1 shows plasma glucose (Glc) levels and plasma fractional enrichment obtained by both infusion protocols. After an initial increase in plasma glucose level during the bolus injection, the level dropped within 20 minutes to a hypoglycemic level of 2.8-3.0 mM. With a bolus of 50% 13C-1-Glc, the fractional enrichment still increased up to 50 min. However, with a bolus of 100% 13C-1-Glc, the fractional enrichment was already at a level of 40% at t = 5 min., then dropped to 33% at t = 20 min. and rapidly increased again to the equilibrium value of 39-40%. Both infusion protocols resulted in very similar equilibrium values. Figure 2 clearly shows signal increases of all important Glc metabolites in 20-min. 13C MR spectra, obtained under hypoglycemic conditions using infusion protocol B.

Discussion and conclusion

This study shows that also under HYPOglycemic conditions high-quality time-dependent MR signal increases of 13C-labeled brain metabolites can be obtained. These data can be used as input for model functions [6] to obtain information on human brain metabolism during hypoglycemia. Infusion protocol B is preferred above protocol A, as B results in minimal variation of fractional 13C enrichment of the plasma Glc. Although 50% 13C-1-Glc was infused during both clamps, the equilibrium fractional enrichment did not exceed 39-40% due to counterregulation-driven endogeneous glucose raising action. For that reason, we advise infusion with 40% 13C-1-Glc during a normoglycemic clamp when brain metabolism during normoglycemia is compared with hypoglycemia using 50% 13C-1-Glc infusion. This method not only allows studies on normal brain metabolism during hypoglycemia, but it also opens possibilities to study differences in hypoglycemic brain metabolism of diabetic patients with and without hypoglycemia unawareness.

References: 1. Gerich JE, et al., Endocr Rev 12 (1991), 356-371**. 2.** Gruetter R, J Neurosci Res 74 (2003), 179-183; **3.** Boyle PJ, et al., Proc Natl Acad Sci USA 91 (1994), 9352-9356; **4.** De Galan BE, et al., Diabetes 51 (2002), 790-796; **5.** Klomp DWJ, et al., Magn Reson Med, in press; **6.** Gruetter R, et al., Am J Physiol Endocrinol Metab 281 (2001), E100-E112.

Acknowledgments: We thank Karin Saini and Bas Schouwenberg for assistance during the hyperinsulinemic hypoglycemic clamps and the Dutch Diabetes Research Foundation (Grant 2004.00.012) and NIH (Grant R21 DK069881) for financial support.