Introduction

During hypoglycemia the supply of the brain with its primary energy substrate, glucose, is reduced. The metabolic effects of this condition have been examined by 1H MRS under hypoglycemia [1] or after several hypoglycemic periods [2]. Studies under hypoglycemia have been done by 13C MRS as well, but with alternative substrates like acetate [3] and lactate [4]. Thus the direct effect of hypoglycemia on cerebral glucose metabolism remains unclear. Aim: to study the direct effects of hypoglycemia on cerebral glucose metabolism by 13C MRS with the infusion of [1-13C]glucose. This involved: a) the registration of 13C-label from glucose into isotopic positions in downstream amino acids (e.g. glutamate-C4 and C3) and b) modeling of this data by a simple one-compartment model [5] to estimate metabolic kinetics in the brain. The model included 13C labeling of plasma lactate and exchange with 13C labeled brain lactate.

Materials and methods

Eight healthy volunteers (4 male/4 female, 23.2±2.5 yrs old) were subjected to two hyperinsulinemic (60 mU·m-2·min-1) glucose clamps after an overnight fast [6]. They were clamped at euglycemia (~5mmol/L) on one day and at hypoglycemia (~3mmol/L) on another day, 4 weeks apart. The clamps were designed to have stable and comparable levels of [1-13C]glucose in plasma. They started with a bolus of 6 g of 100% [1-13C]glucose 20% (w/w) solution infused over 10 minutes to increase plasma glucose 13C enrichment followed by two hours by infusion of respectively 40% and 50% enriched [1-13C]glucose at euglycemia and hypoglycemia at a variable rate to maintain target plasma glucose levels. Arterial blood was sampled in 5 min intervals to determine plasma glucose concentration and [1-13C]glucose isotopic enrichment by high resolution 1H NMR. For in vivo measurements a DEPT sequence was used in combination with ISIS localization and 1H decoupling. 13C-MRS acquisition (72 scans, TR=2s, duration=2.5 min) of a voxel of ~125 ml in the occipital brain tissue 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 1H with a CP surface coil insert for 13C [7]. The FIDs of 8 reference spectra were averaged and subtracted from all FIDs to remove natural abundance signals and baseline distortions due to residual lipid signals. To enhance SNR the FIDs were added in running averages of 15 min (6 spectra).

These spectra were fitted in jMRUI with the AMARES algorithm. For quantification the natural abundance 13C Myo-inositol signal was assumed to reflect a tissue level of 6 μmol/g. In addition, the data were corrected for the acquisition sequence pulse profiles (measured in a phantom).

Modeling was performed with a standard one-compartment model as previously used in [5], where plasma glucose and lactate C3 being labeled by 5% per hour (see Fig 2, solid and dotted lines) during euglycemia (closed symbols/solid lines) and hypoglycemia (see Fig 2). During the first 20 min of the experiments the clamp procedure 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 1H with a CP surface coil insert for 13C [7]. The FIDs of 8 reference spectra were averaged and subtracted from all FIDs to remove natural abundance signals and baseline distortions due to residual lipid signals. To enhance SNR the FIDs were added in running averages of 15 min (6 spectra).

Further assumptions for the modeling were [Glutamate]brain =10 μmol/g, [Glutamine]brain =4 μmol/g, this results in a concentration of 0.1 μmol/g [3-13C]lactate in plasma after two hours. Further assumptions for the modeling were [Glutamate]brain =10 μmol/g, [Glutamine]brain =4 μmol/g.

Results

In glutamate 13C label appears first at the C4 and then at the C3 position both under euglycemia and hypoglycemia (see Fig 2). During the first 20 min of the experiments the clamp procedure was similar for both glucose conditions, resulting in comparable glutamate curves. Later in the experiment the 13C enrichment in plasma glucose starts to deviate between groups, causing lower percentage enrichment during hypoglycemia (30%) versus euglycemia (35%) even though a higher glucose enrichment was used for infusion during the hypoglycemic experiment to compensate for less total glucose infusion under this condition. The experimental curves are well fitted by the one-compartment model, when assuming plasma lactate C3 being labeled by 5% per hour (see Fig 2, solid and dotted lines for eu- and hypo hypoglycemia respectively). The corresponding flux values (in μmol/min/g) through the Tricarboxylic acid (TCA) cycle for the euglycemic and hypoglycemic experiment respectively are V_{TCA} =0.58 and 0.54.

Discussion and conclusion

Volunteers in this study suffered from clear hypoglycemic symptoms. Nevertheless, under the assumption of identical labeling patterns of [3-13C]lactate in plasma, V_{TCA} values are comparable for eu- and hypoglycemia. This indicates that normal glucose metabolism in the brain is maintained and hypoglycemia as applied in this study does not affect major brain functions. There are several explanations for this outcome; first of all the induced hypoglycemia is mild, it can be that even though the rest of the body is alarmed by a glucose deficiency, this is not a rate limiting concentration for brain metabolism. Furthermore, these volunteers are aware of the hypoglycemia, this awareness possibly increases brain activity in the occipital region, and therefore V_{TCA} is maintained during hypoglycemia in this brain region [9]. These results are also in agreement with studies on rats [10], where direct hypoglycemia did not influence cerebral glucose metabolism in control animals.