Observation of label accumulation at Glutamate/Glutamine $C_{1,2,3,4}$ and HCO_3^- in human brain after intravenous 1-^{13}C labeled Glucose infusion

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Introduction:
Glucose (Glc) is the major respiratory fuel of the human brain generating 36 ATP's per molecule if completely oxidized to CO_2 + H_2O. Decarboxylation steps include pyruvate dehydrogenase, isocitratedehydrogenase, and oxoglutarate dehydrogenase. Proof of complete oxidation comes from the detection of CO_2 (or HCO_3^-) formation. Several groups demonstrated that once intravenously (i.v.) infused 1-^{13}C Glc passes the blood-brain barrier label accumulation in glutamate (Glu), glutamine (Gln), γ-amino butyric acid (GABA), lactate (Lac), and aspartate (Asp) can be detected (1-7). These experiments in humans did not allow the observation of signals resonating at > 150 ppm and enrichment of HCO_3, Glu_1 and Gln_1, as shown in tissue slices and extracts of animal brain (8,9), was not reported.
Aims: (i) Establish assignments and enrichment patterns in human brain after Glc infusion for resonances at chemical shifts > 150 ppm. (ii) Demonstrate CO_2 production from glucose oxidation *in vivo*.

Material and Methods:
Two adult controls (male 27 years, fed, female 29 years, fasted) were studied with natural abundance and with ^{13}C MRS after i.v. glucose infusion using a protocol modified from that developed by DeFronzo et al. (10) by omitting somatostatin. Experiments were carried out on a GE, Signa 1.5 T system using a coil design as described previously (11,12). A baseline spectrum and spectra during infusion were acquired from the occipital brain region with a FID sequence, TR = 1s, 1024 pts, 4 kHz excitation bandwidth.

Results:
As in earlier studies, labeling of $Glu_{2,3,4}$, $Gln_{2,3,4}$, $Asp_{2,3}$, and $NAA_{2,3}$ was observed (Fig. 1). In the region > 150 ppm the chemical shifts of the ^{13}C enriched resonances at 175.3 and 174.8 ppm, are consistent with Glu_1 and Gln_1, the resonance at 160.9 ppm is consistent with carbonate (HCO_3^-) (Fig. 2). ^{13}C enrichment of these resonances was observed in both subjects after ≈ 50 minutes.

Discussion:
These findings represent an extension of previous studies (*4-6*) where enrichment of Glu_1, Gln_1, and HCO_3^- was not reported. Our data suggest that proton decoupled ^{13}C MRS allows a more detailed definition of the fates of 1-^{13}C glucose carbons, including the accumulation in Glu_1, Gln_1, and HCO_3^-, than previously available. This potentially will provide a more precise simultaneous determination of the TCA-cycle flux rate, glutamine synthesis rates, malate-aspartate shuttle exchange rate, α-ketoglutarate/glutamate exchange rates, now including substrate decarboxylation and total glucose oxidation in normal and diseased human brain.

References

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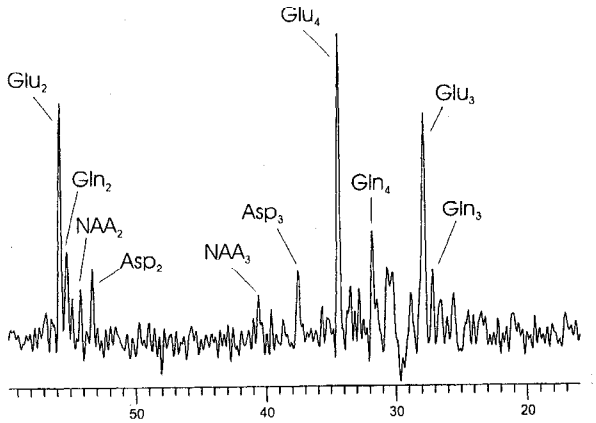

Fig. 1: A difference spectrum, generated by subtracting the baseline scan from the spectrum acquired between 75-145 min after infusion start from the male subject, shows label accumulation in various amino acid resonances between 20 - 60 ppm chemical shift.

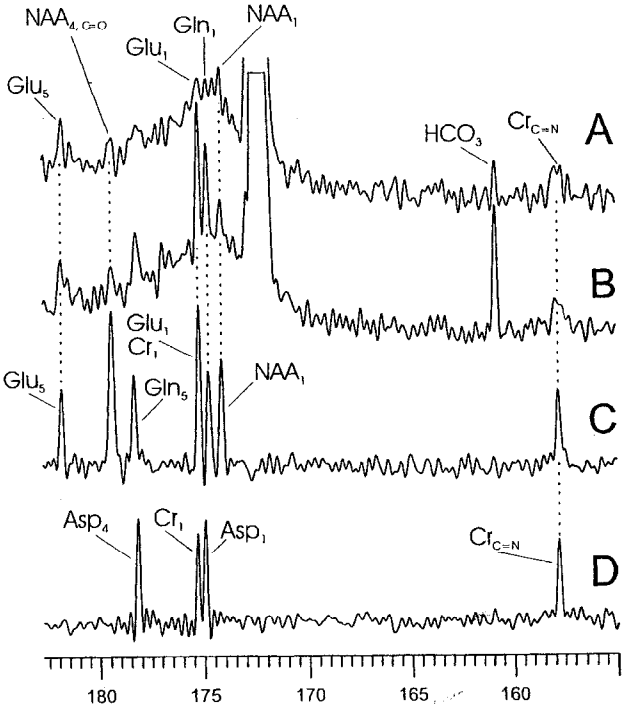

Fig. 2: Due to the large excitation bandwidth of 4 kHz (≈ 200 ppm) signal from metabolites resonating at > 150 ppm can be observed simultaneously. Shown is the baseline spectrum (A), acquisition time 25 min, and the spectrum acquired between 140 - 180 min (B), aligned with spectra from model solutions of Glu, Gln, NAA, and Cr (C), and Asp and Cr (D) from the female volunteer. A ≈ 4% enrichment of HCO_3 can be observed. Two resonances consistent with Glu_1 and Gln_1 are also clearly enriched.

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