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Introduction

^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy has been recently established as the only method to measure brain glycogen non invasively in a reliable fashion (ref. 1). However, all ^{13}C NMR studies of brain glycogen to date relied on observing the incorporation of ^{13}C label to overcome sensitivity limitations. Given that only the ^{13}C -labeled part of the glycogen molecule is measured, we sought to develop a method allowing to measure absolute glycogen concentration using *in vivo* NMR. The rationale for this method was to achieve near steady-state enrichment using long-term ^{13}C -labeled glucose (Glc) administration and to calculate this enrichment *in vivo* using NAA as a reporter molecule, because NAA has a similar turnover time as glycogen (ref. 2). A second aim was to evaluate the rate of ^{13}C glycogen turnover in activated conscious rats.

Methods

Male Sprague-Dawley rats (n=17, 237.5 ± 6.9 g, average ± SEM) were fasted overnight with free access to water. Then, a 10% [^{13}C]-labeled Glc solution was their sole source of exogenous carbon for ~ 24 hrs (group A, n= 7) or 48 hrs (group B, n = 6). Then, the rats were anesthetized using 2% isoflurane anesthesia and placed into the *in vivo* magnet (9.4 Teslas) for indirect measurement of ^{13}C -NAA enrichment using ACED-STEAM (ref. 3) and localized detection of ^{13}C -glycogen (fig. 1). At the end of each experiment, rats were sacrificed using a 4kW focused microwave fixation device (1.4 sec). Brains were dissected and glycogen assay was performed on the 0.03N HCl brain extracts to investigate brain glycogen and Glc concentrations (ref. 4). Then ^{13}C enrichment of NAA (fig. 1) and Glc (as well as digested glycogen) were measured *in vitro* by high field spectroscopy (14.1 T, 600 MHz) (ref. 5).

The activated conscious rats were stimulated by regularly putting new tools into the cage to stimulate them for seeking their environment. They had access to ^{13}C -Glc for 4.4 ± 0.4 hrs (time zero = time when they started to drink). Then, they were sacrificed and their brain was processed for *in vitro* analysis of ^{13}C glycogen and NAA enrichment.

Results and discussion

Our results showed that the *in vivo* measurement of the isotopic enrichment (IE) of NAA was within experimental error identical to the *in vitro* measurement (n=9, $R^2=0.95$). Moreover, the NAA IE was highly correlated with the amount of ^{13}C -Glc ingested (n=13, $R^2=0.88$, not shown). After 24 or 48 hours of ^{13}C -label administration, glycogen IE was 2.2 ± 0.2 fold that of NAA (fig. 2), as expected from the breakdown of [^{13}C]-Glc into two molecules of acetyl-CoA. Therefore, at isotopic near steady-state enrichment, the IE of NAA reflects the IE of glycogen. From the IE of glycogen and the ^{13}C quantification of C1 glycogen (fig. 1C) the quantification of brain glycogen concentration was possible.

The quantification of the IE of glycogen from the IE of NAA was based on their similar turnover times reported in the anesthetized rat. To determine if adrenergic stimulation affect total brain glycogen content and turnover we sought to compare glycogen IE with that of NAA after a short period of stimulation (~4 hrs) and ^{13}C administration. NAA IE was 5.7 ± 1.5% consistent with label ingested (3.2 ± 0.6 g), labeling duration and NAA turnover time, whereas brain glycogen IE was 25.2 ± 5.6 % (figure 2, open triangles). Although glycogen total concentration (4.5 μmol/g) was not significantly different than control values (P=0.9, n=15), glycogen IE was more than 5 fold higher than that of NAA IE implying a turnover time which was more that 2 fold faster than reported for resting animals.

We conclude that steady-state glycogen enrichment can be inferred *in vivo* from that of NAA after using long-term ^{13}C -Glc administration.

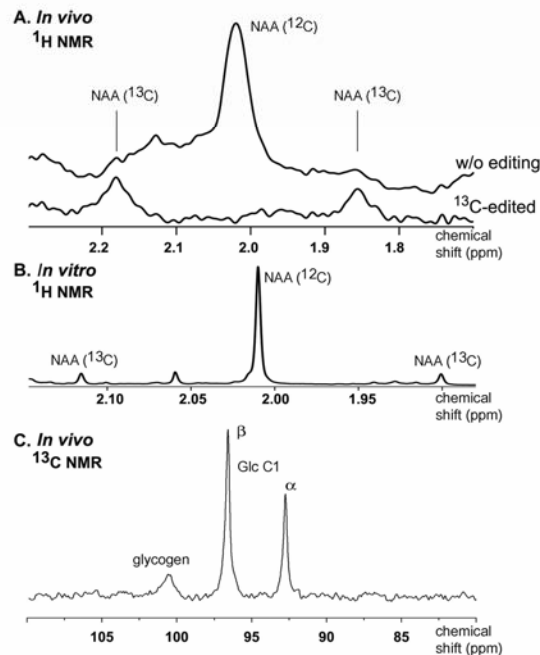


Fig. 1: Examples of decoupled (^{13}C) ^1H NMR detection of $^{13}\text{C}_6$ NAA *in vivo* (A), and in brain extracts (B), as well as the localized detection of C1 of glycogen by ^{13}C NMR *in vivo* (C).

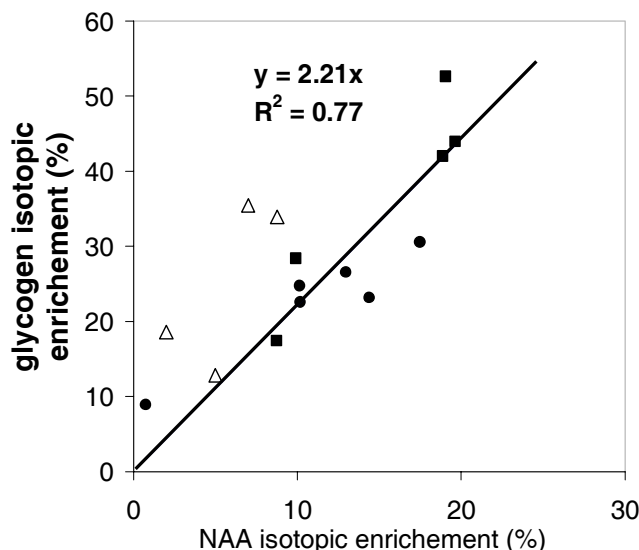


Fig. 2: *In vitro* measurement of ^{13}C -glycogen IE as a function of ^{13}C -NAA IE. The line describe a strong correlation of ^{13}C -glycogen IE as a function of ^{13}C -NAA IE after 24.6 ± 0.4 (solid squares) or 49.7 ± 0.9 hours (solid circles) of ^{13}C -labeled-Glc administration. The open triangles represent the values obtained with the activated rats.

References

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