Direct quantification of *total* brain glycogen concentration during hypoglycemia

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Introduction

Glucose (Glc) is the main substrate for brain energy metabolism. Using ^{13}C NMR spectroscopy in conjunction with infusion of ^{13}C labeled Glc (1, 2), we previously reported that brain glycogen can serve as an energy reserve during hypoglycemia (3). All NMR studies of brain glycogen to date were performed in conjunction with observing the incorporation ^{13}C label into brain glycogen. This represents an experimental complication since ^{13}C label turnover can affect quantitatively the interpretation of the data. The goal of this study was to measure *total* brain glycogen concentration either during acute hypoglycemia or during normoglycemia using biochemical methods (4) and to compare with the ^{13}C NMR results.

Methods

Male Sprague-Dawley rats (273 ± 8 g) were fasted overnight with free access to water. They were anesthetized using 2% isoflurane, and then intubated and ventilated during the rest of the experiment. Catheters were inserted into both femoral arteries for blood gases and Glc analysis, and into both femoral veins for intravenous infusion of α-chloralose, Glc and insulin. After surgery, anesthesia was switched to α-chloralose (26.7 mg/kg/hr). At the end of each experiment, rats were sacrificed using a 4kW focused microwave fixation device (1.4 sec). Brains were dissected and a glycogen assay was performed on the 0.03N HCL brain extracts (4). Lactate measurements of these extracts (mean lactate ± SEM: 1.52 ± 0.11 μM/g) allowed to directly assess postmortem degradation effects.

6 groups of animals were studied:

- **(A)** Rats maintained for 8 hrs under α-chloralose anesthesia (control rats, n=5).
- **(B)** Rats maintained at ~10 mM plasma Glc for 3 hrs before acute hypoglycemia induction using ~12 insulin unit/kg/hr. Hypoglycemic state (< 2mM plasma Glc) for 30 min (short hypoglycemia, n=5).
- **(C)** Same as B, hypoglycemia lasted for 2 hrs (long hypoglycemia, n=6).
- **(D)** Rats fasted overnight and anesthetized using isoflurane prior to sacrifice. No surgery (fasted only, n=4).
- **(E)** Rats fasted overnight, and then fed ad libitum for 48 hrs with a 10% Glc solution only, as described previously (5). (48 hrs 10% Glc, n=6).
- **(F)** Same as E, brain glycogen was obtained by ^{13}C NMR (5).

Results and discussion

At plasma Glc levels lower than 2 mM (mean ± SD: 1.59 ± 0.19 mM), the brain Glc concentration was 0.08 ± 0.05 μmol/g (Fig. 1), consistent with Glc transport becoming rate limiting for whole brain metabolism. Insulin-induced hypoglycemia promoted gradual glycogenolysis at a rate of ~1.5 μmol/g/hr even though brain glucose was similar in both hypoglycemic groups (fig. 1: B & C). These results suggest that *total* brain glycogen serves as a viable glucose reservoir during extended periods of hypoglycemia.

In contrast, overnight fasting did not substantially affect brain glycogen (fig. 2, compare groups D & E), consistent with brain Glc not being rate-limiting for metabolism in fasting. The biochemical measurement of brain glycogen was in excellent agreement with ^{13}C NMR measurements (fig. 2, compare groups E & F).

Conclusion

We conclude that previous interpretation of ^{13}C NMR spectroscopy data (3, 5, 6) accurately reflected the changes in *total* brain glycogen content. Brain glycogen thus provides an aspect of *in vivo* cerebral carbohydrate metabolism that can serve as a substantial source of glucosyl units during insulin-induced hypoglycemia (7).

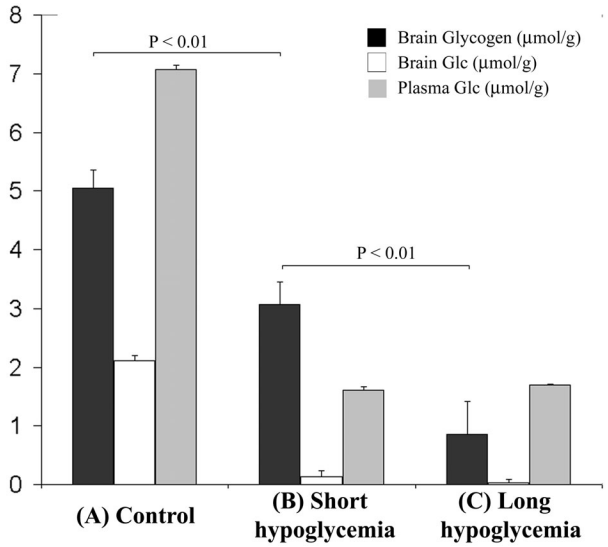

Fig. 1. Extended durations of hypoglycemia are necessary to deplete brain glycogen. Although the decrease of brain glycogen after only 30 min of hypoglycemia is significant (P<0.01), glycogen decreases further after 2 hours as compared to 30 min hypoglycemia (P<0.01). Plasma Glc represent the averaged values obtained during (A) 8 hrs, (B) 30 min and (C) 2 hrs. Error bars indicate SEM.

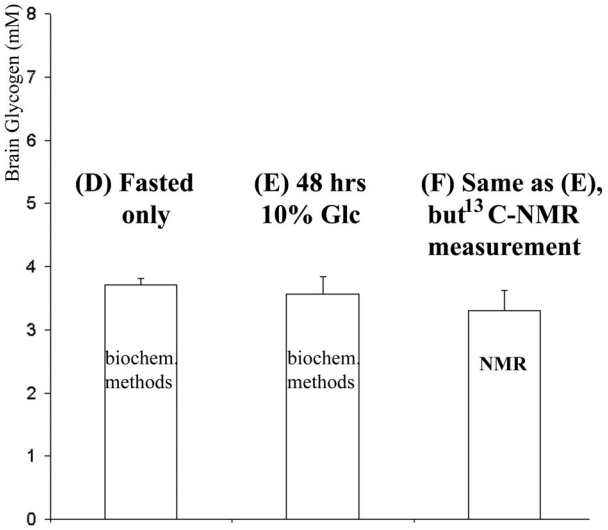

Fig. 2. Direct comparison of brain glycogen measurement using biochemical methods as compared to ^{13}C NMR. Feeding the rats for 48 hrs with a 10% Glc solution had no discernible effect (compare groups D and E). Error bars indicate SEM.

Acknowledgements

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