Localized ^{13}C NMR spectroscopy of [1-^{13}C] brain glycogen recovery following a single episode of hypoglycemia

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INTRODUCTION

Glycogen is an endogenous carbohydrate store for energy metabolism in many mammalian tissues, and can be utilized when glucose supply is limited. Recent studies have suggested that brain glycogen is an important store of glucose equivalents for the brain and provides the majority of the fuel deficit during acute insulin-induced hypoglycemia (1). Following hypoglycemia, rat brain glycogen increased to levels higher than before the hypoglycemic episode (supercompensation or rebound) (1). The question remained as to whether the increase in glycogen resynthesis persisted for longer time periods than were measured in that study (1). The purpose of this study was to measure prolonged brain glycogen resynthesis after hypoglycemia for up to 14 hours.

METHODS

Seven Sprague-Dawley male rats (250±10g, mean±SD) were fasted overnight before the experiments. After vascular access was secured under 2% isoflurane anesthesia, α-chloralose (24-26mg/kg/hr) and 20% weight/volume of 99%-enriched [1-^{13}C] glucose (0.1ml/hr) infusions were started. 30-60 min later, a single episode of hypoglycemia was introduced by an i.v. infusion of insulin (30-70 IU/kg). Plasma glucose levels were measured in a glucose analyzer (Analox GM7) every 10 min during hypoglycemia and every 30 min thereafter. Plasma glucose was kept below 2 mM for ~2 hours of hypoglycemia. Following this hypoglycemic episode, the [1-^{13}C]glucose infusion rate was increased to restore plasma glucose to ~14mM for 13±1 hours. Physiology was normal as assessed from arterial blood gases (pCO_2=39.6±2.1 mmHg, pH=7.39±0.02, pO_2>100 mmHg, temperature=37.1±0.1). 2 to 3 hours after restoring glycemia, ^{13}C NMR measurements of brain glycogen C1 and glucose were performed in a 9.4T/31cm magnet using OVS localized ^{13}C MRS from a 500 μl volume (1). All NMR data were corrected for NOE and loading, and quantified using a phantom reference at 37°C.

RESULTS AND DISCUSSION

Hypoglycemia resulted in arterial plasma glucose concentrations of 1.6±0.1 mM (n=7). Rat brain glycogen C1 increased in a close to linear manner in all rats for 13±1 hours after the hyperglycemic episode (Fig. 1). In some animals, brain glycogen C1 continued to increase beyond 14 hours (Fig. 2). The current study extends the measurement of brain glycogen to longer time periods following a single hypoglycemic episode. Given that in the awake rat, brain glycogen metabolism is comparable to that of NAA (2), a restoration of brain glycogen concentration to normal may require days or weeks. Recent evidence suggested that human brain glycogen metabolism is several fold slower than in rats (3). It is hypothesized that the extended time required for brain glycogen rebound in the rat may be even longer in the human brain. Together with the slow glycogen turnover, the restoration of brain glycogen may take weeks in human, which is also the time required to restore hypoglycemia awareness in type I diabetes (4).

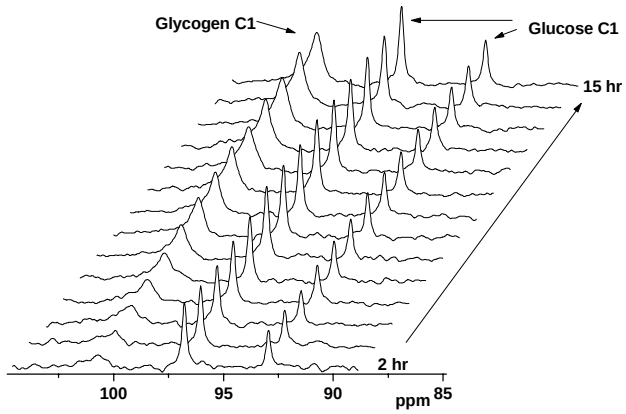

Figure 1 Time-resolved observation of cerebral glycogen C1 labeling. Shown is the time course of the [1-^{13}C]glycogen and α, β [1-^{13}C]glucose signals. Time 0 represents the start of hyperglycemia. Each spectrum corresponds to ~1 hour of data accumulation (line broadening 20 Hz).

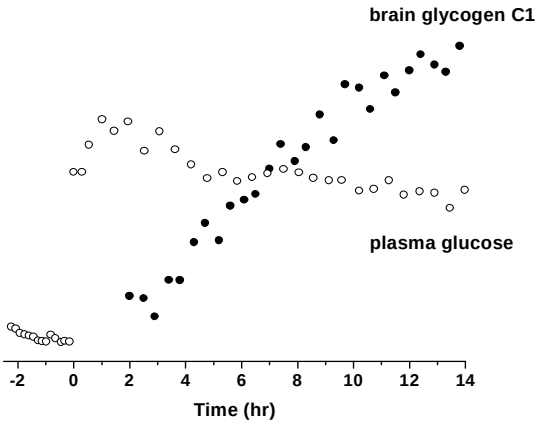

Figure 2 Time-resolved observations of cerebral glycogen C1 labeling (closed circles) and plasma glucose (open circles). Time –2 to 0 indicates hypoglycemia period. The data shown are from the same animal as Fig. 1.

REFERENCES

1. IY Choi, et al., *J Neurosci Res* **72**: 25 (2003)
2. IY Choi, et al., *Neurochem Int.* **43**: 317(2003)
3. G Oz, et al., *Neurochem Int* **43**: 323 (2003)
4. PE Cryer, et al., *J Cereb Blood Flow Metab* **21**: 483 (2002)

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