Chronic hypoglycemia increases the brain glucose concentration: A ^{13}C NMR study

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Introduction

Glucose is the main source of energy for brain function. Recently it has been reported that glycogen may serve as an important store of glucose equivalents in the brain during acute, insulin-induced hypoglycemia (1). Glycogen has been further implicated in the mechanism causing a lowered glycemic threshold for counterregulation ("Hypoglycemia Unawareness"), observed in patients with diabetes following a *single* episode of hypoglycemia (1). Following recurrent antecedent hypoglycemia, similar reductions in the threshold for counterregulation have been observed. However, during *chronic* hypoglycemia, the limited brain glycogen stores are likely to be inadequate to sustain the glucose supply deficit. Studies have reported that increased uptake of glucose and glucose transporter expression is a result of *chronic* hypoglycemia (2). Elevated capacity of glucose transport into the brain is expected to result in higher brain glucose concentrations. Therefore, the purpose of this study was to measure brain glucose and glycogen concentrations in rats subjected to *chronic* hypoglycemia using localized ^{13}C MRS (3).

Methods

Hypoglycemia was induced on day 0 in seven male Sprague-Dawley rats (176±11g, Harlan) by subcutaneous implantation of insulin implants (6-8 IU/day) as described as elsewhere (2). Animals were considered hypoglycemic when morning plasma glucose values were < 3 mM, as determined by tail bleeds (average plasma glucose was 2.5±0.2 mM (n=7)).

All *in vivo* NMR studies were performed at 9.4 T between day 12 and 14. Animal was prepared and anesthetized under α-chloralose (26.7mg/kg/hr) as described (3). Localized ^{13}C NMR signals of glucose and glycogen were measured in a 440 µl volume of the rat brains using OVS localization (3). Physiology (temperature, arterial pH, pCO_2, pO_2 and BE) was maintained within normal range. Plasma glucose was measured throughout the measurement and the stability of brain glucose was assessed from the ^{13}C NMR. Brain glucose and glycogen concentrations were obtained when brain and plasma glucose levels were stable for over 20 min. NMR signals were corrected for loading and NOE effect, and quantified by phantom reference studies at 37°C. Immediately following the NMR experiments, rat brains were fixed using focused microwave (4), extracted and prepared for glycogen and glucose assay (5).

Results and Discussion

Insulin-induced chronic hypoglycemia resulted in significantly elevated brain glucose content, when compared to normal (Fig. 2). The increased brain glucose concentrations are consistent with previous in situ perfusion studies and assessment of glucose transporter expression in the rat (2). [1-^{13}C] brain glycogen concentrations measured by ^{13}C NMR after 9±1 hrs of ^{13}C glucose infusions were 3.2±0.7 µmol/g (n=7), comparable to that measured in one rat using biochemical assay. Brain glycogen concentrations were comparable to those measured using biochemical extraction following overnight fast (4.0±0.4 µmol glucosyl units/g, n=8) and imply little effect of chronic hypoglycemia on brain glycogen content.

In conclusion, the elevated brain glucose concentrations can explain the lowered threshold for counterregulation observed in *chronic* hypoglycemia.

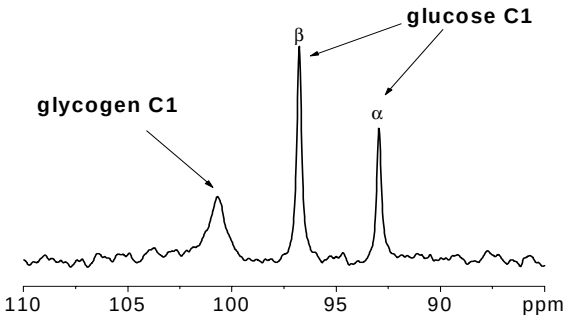

Figure 1 Localized ^{13}C NMR of glucose and glycogen in one of the current chronic hypoglycemia studies (20Hz line broadening)

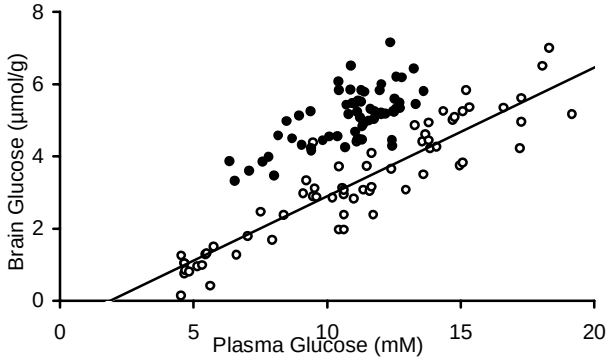

Figure 2 Brain glucose concentrations in chronic hypoglycemia (closed circles) compared to normal (open circles, from Ref 3)

References

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