Glucose Transport Kinetics Upregulates in Chronic Hypoglycemic Rats: An in vivo ^{13}C MRS Approach

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Introduction

Glucose is the major energy source to maintain normal brain function. Glycogen has recently been proposed as an important fuel reservoir in the brain during acute hypoglycemia and increased brain glycogen stores have been considered in the mechanism causing hypoglycemia unawareness (1). However, during chronic hypoglycemia, the limited brain glycogen stores are likely to be inadequate to sustain the glucose supply deficit. Instead, chronic hypoglycemia results in an increased uptake of glucose and glucose transporter expression (2). Such GLUT-1 transporter upregulation in BBB is expected to result in higher brain glucose concentrations. Preliminary data indicated increased brain glucose concentrations compared to published brain glucose concentrations (3, 4). The aim of the present study was to extend these measurements to compare the brain glucose transport kinetics measured in and after chronic hypoglycemia to those in sham-operated animals, and to validate the NMR quantification of glucose.

Methods

In 17 rats (~180g, male Sprague-Dawley), chronic hypoglycemia (morning plasma glucose was 2.5 ± 0.2 mM) was induced by subcutaneous implantation of insulin implants (6-8 IU/day) for 12-14 days (2, 3). Sham-operated animals (n=13) were treated in exactly the same manner (including morning tail bleeds), except that the pellets did not contain insulin and animals were fasted overnight prior to the NMR measurement. Both groups were prepared under 2% isoflurane and which was switched to α-chloralose (~26.7 mg/kg/hr). Localized [1-^{13}C] brain glucose concentrations were measured in ~440 µl volumes using OVS method (4). Arterial blood was sampled to maintain physiological condition within normal range and access plasma glucose throughout the measurement and the stability of brain glucose was assessed by ^{13}C NMR. Brain glucose NMR signals were quantified by phantom reference studies at 37°C (4). Immediately following the NMR experiments, rat brains were fixed using focused microwave (4 kW, 1.4 sec), carefully extracted and biochemically assayed (5). To achieve brain glucose measurements at low plasma glucose concentrations, (n=5) animals were analyzed using biochemical assay without NMR measurements. Brain glucose and plasma glucose were fitted by the reversible Michaelis-Menten model (6).

Results and Discussion

Chronic hypoglycemia resulted in significantly elevated brain glucose content compared to the sham-operated group (Fig 1). Biochemical determination of brain glucose content was within 10% of that measured by NMR (Fig 1). Fitting the reversible Michaelis-Menten model resulted in an apparent Michaelis-Menten constant of K_t = 3.3 ± 1.0 mM and an apparent relative maximal transport rate of T_{max}/CMR_{glc} = 4.2 ± 0.2 in the chronic hypoglycemic animals; and K_t = 3.3 ± 1.4 mmol/L and T_{max}/CMR_{glc} = 2.7 ± 0.2 in the sham-operated group. The 55 ± 11% ($p<0.001$) increase in T_{max} is in the range as reported 26-42% increase in cortical GLUT-1 protein expression (2). It was of interest to note that, in chronic hypoglycemic rats, brain glucose concentration was normal at a hypoglycemic plasma glucose level of 2-3 mM.

Considering the experimental precision of the present study and the previous transporter studies, we conclude that it is likely that increases in T_{max} account for most of the brain glucose content increase, mediated by an increase in the number of transporter proteins at the blood-brain barrier. This is the first study corroborating the physiology of brain glucose homeostasis with glucose transporter protein expression.

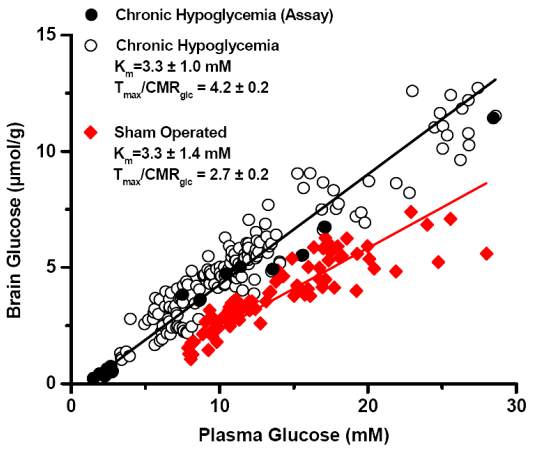

Figure 1. Linear relationship between plasma and brain glucose. Black open circles represent brain glucose concentrations by NMR data and closed circles by assay. Diamonds indicate NMR data from sham-operated group. The fits of the reversible Michaelis-Menten model are indicated by the straight lines.

Références

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