Brief Communication

MOLECULAR METABOLISM

Regional differences in brain glucose metabolism determined by imaging mass spectrometry

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ABSTRACT

Objective: Glucose is the major energy substrate of the brain and crucial for normal brain function. In diabetes, the brain is subject to episodes of hypo- and hyperglycemia resulting in acute outcomes ranging from confusion to seizures, while chronic metabolic dysregulation puts patients at increased risk for depression and Alzheimer's disease. In the present study, we aimed to determine how glucose is metabolized in different regions of the brain using imaging mass spectrometry (IMS).

Methods: To examine the relative abundance of glucose and other metabolites in the brain, mouse brain sections were subjected to imaging mass spectrometry at a resolution of 100 μm. This was correlated with immunohistochemistry, qPCR, western blotting and enzyme assays of dissected brain regions to determine the relative contributions of the glycolytic and pentose phosphate pathways to regional glucose metabolism.

Results: In brain, there are significant regional differences in glucose metabolism, with low levels of hexose bisphosphate (a glycolytic intermediate) and high levels of the pentose phosphate pathway (PPP) enzyme glucose-6-phosphate dehydrogenase (G6PD) and PPP metabolite hexose phosphate in thalamus compared to cortex. The ratio of ATP to ADP is significantly higher in white matter tracts, such as corpus callosum, compared to less myelinated areas. While the brain is able to maintain normal ratios of hexose phosphate, hexose bisphosphate, ATP, and ADP during fasting, fasting causes a large increase in cortical and hippocampal lactate.

Conclusion: These data demonstrate the importance of direct measurement of metabolic intermediates to determine regional differences in brain glucose metabolism and illustrate the strength of imaging mass spectrometry for investigating the impact of changing metabolic states on brain function at a regional level with high resolution.

Keywords Brain imaging; Glucose metabolism; Pentose phosphate pathway; Glycolysis; ATP; Mass spectrometry

1. INTRODUCTION

Glucose is the main energy substrate for the brain and accounts for about 20% of whole-body glucose utilization [1—3]. People with diabetes may have periods of hyperglycemia or hypoglycemia, and these changes in glucose can be associated with brain dysfunction ranging from confusion to seizures. In addition, the increased long-term risks of depression and Alzheimer's disease associated with diabetes [4] highlight the importance of insulin action and metabolic status on short- and long-term brain function and survival.

Glucose is metabolized in cells either by glycolysis or the pentose phosphate pathway (PPP). In the glycolytic pathway, glucose is converted to pyruvate which can be converted to lactate by lactate dehydrogenase or sent to the tricarboxylic acid (TCA) cycle to generate ATP via oxidative phosphorylation. Alternatively, glucose can enter the PPP via the action of glucose-6-phosphate dehydrogenase (G6PD) to generate 5-carbon sugars, as well as NADPH, which is a co-factor for cholesterol biosynthesis and protects against oxidative stress [5]. Though each cell can use glucose for either glycolysis or the PPP, different cell populations favor distinct metabolic pathways [6,7].

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The mouse brain consists of roughly 70% neurons and 30% non-neuronal cells with different brain regions exhibiting distinct compositions of cells [8]. Neurons and astrocytes metabolize glucose mainly via glycolysis for the generation of ATP while oligodendrocytes must generate the cholesterol rich myelin sheaths. Thus, oligodendrocytes shuttle more glucose into the PPP in order to generate NADPH for cholesterol biosynthesis [9]. It has been estimated that 10% of glucose in oligodendrocytes is used for the PPP [10], but this apparently small proportion of glucose may be higher as the PPP seems to be underestimated in comparison to glycolysis by many approaches [11].

To investigate the regional fate of glucose, we assessed multiple steps within the glycolytic and PPP using a combination of gene and protein expression, protein activity assays, and imaging mass spectrometry (IMS). We demonstrate that while measurements of enzyme expression and activity point to differences in the regional activity of glycolysis and the PPP, IMS provides a direct measurement of the metabolites generated in these pathways in specific brain regions, including those which are otherwise difficult to assess, such as the fimbria or corpus callosum. Further, we demonstrate that in these highly myelinated white matter tracts there is a high ATP/ADP ratio but not a similarly high hexose bisphosphate/hexose monophosphate ratio, supporting the notion that the lactate shuttle may be very important for energy metabolism in these brain regions. Thus, IMS provides a powerful tool for high resolution assessment of glucose metabolism across brain regions.

2. MATERIALS AND METHODS

2.1. Animals

All mice were housed in a mouse facility on a 12 h light/dark cycle in a temperature-controlled room. 10–12 week old male C57Bl/6J mice (Jackson Laboratories: stock nr. 000664) were maintained on a standard chow diet (Mouse Diet 9F 5020; PharmaServ). When fasted, animals had free access to water. Animal care and study protocols were approved by the Animal Care Committee of Joslin Diabetes Center and were in accordance with the National Institutes of Health guidelines as well as the animal welfare committees of the German Institute of Human Nutrition (DIfE) and the local authorities (LUGV, Brandenburg, Germany).

2.2. Immunostaining

Mice were anesthetized with an intraperitoneal (i.p.) injection of Avertin (300 mg/kg) and transcardially perfused with PBS followed by 4% paraformaldehyde. Brains were dissected and post-fixed in 4% paraformaldehyde overnight, cryoprotected in 15% (w/v) then 30% sucrose, and frozen in OCT compound (Tissue-Tek). Serial coronal 30 μm sections were washed, blocked (0.2% Triton-X-100 + 5% normal goat serum (NGS) in PBS), and stained with primary antibody (Table A.1) diluted in PBS containing 0.1 Triton X-100 + 1% NGS overnight at 4 °C. Sections were then washed and subsequently stained with secondary antibodies (Vectastain VIP, Vector Labs or goat anti-rabbit Alexa Fluor 488 and goat anti-mouse Alexa Fluor 594, 1:500, ThermoFisher Scientific). Sections were mounted with SlowFade Diamond mountant containing DAPI (Life Technologies) and imaged with a 10× objective on a Zeiss LSM710-Duo confocal microscope.

2.3. Analysis of gene expression by quantitative PCR

Total RNA from brain regions was isolated using an RNeasy Mini Kit (Qiagen). 1 μg of total RNA was reverse transcribed in 20 μl using the High Capacity cDNA Reverse Transcription Kit (Promega). 10 ng of synthesized cDNA was amplified with specific primers in a 10 μl PCR reaction using a SYBR green PCR master mix (Promega). Analysis of gene expression was performed using an ABI ViiA™ 7 Real-Time PCR System with 384-Well Block with initial denaturation at 95 °C for 10 min, followed by 40 PCR cycles, each cycle consisting of 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 1 min, and SYBR green fluorescence emissions were monitored after each cycle. For each gene, mRNA expression was calculated relative to TATA binding protein (TBP) expression. Amplification of specific transcripts was confirmed by the melting-curve profiles.

2.4. Enzyme assays

Phosphofructokinase and hexokinase assays were performed according to manufacturer's guidelines (Sigma), and G6PD activity assay was performed as previously described [12].

2.5. Imaging mass spectrometry and MS/MS

Mice were maintained on a chow diet and sacrificed in random order in the morning after a 16 h overnight fast or following ad libitum access to chow. IMS was performed on five individuals from each treatment group and HP, HBP, ATP, and ADP relative abundance were assessed for regions of interest. Serial sections of the brains were used to analyze lactate.

Gold-coated stainless steel MALDI target plates were precoated with α-cyano-4-hydroxycinnamic acid (CHCA) using an automated sprayer (TM Sprayer, HTX Technologies). CHCA was prepared as 5 mg/ml in 90% acetonitrile and was sprayed at 0.15 ml/min at 100 °C at 700 mm/min plate velocity. Four passes were deposited at 2 mm spacing, alternating horizontal and vertical positions between passes with a 1 mm offset for the second passes in each direction. Sections from fresh frozen mouse brain were obtained at 12 μm thickness in a cryostat. The sections were directly thaw-mounted onto the precoated target plates. The tissues were subsequently post-coated with 9-aminoacridine (9AA) matrix, prepared at 5 mg/ml in 90% methanol, and applied using the same program as for CHCA on the TM Sprayer. Sections designated for lactate imaging were only postcoated with 9AA. Metabolite images were acquired on a 9.4T FT-ICR mass spectrometer (Bruker Solarix, Bruker Daltonics) in negative ionization mode at 100 μm spatial resolution. Tentative metabolite identifications were made based on accurate mass, which is typically better than 2 ppm. Masses were searched against the Human Metabolome Database (www.hmdb.ca) for possible identifications as well as possible isobaric interferences. Because there are numerous structures and linkages possible for hexose phosphate and bisphosphate sugars, we categorize them as generic hexose phosphate and hexose bisphosphate species. Table A.2 shows the likely identifications and ion forms of the metabolites imaged in this work. In order to more fully characterize some species, serial sections were prepared as described above and analyzed for select metabolites via MS^3 imaging on a linear ion trap equipped with a MALDI source and a nitrogen laser (Thermo LTQ XL). Images were acquired at 100 μm spatial resolution with a 50 μm spiral raster for each transition. The following transitions were monitored: ATP: MS^3 m/z 506 → m/z 408 → m/z 273 + 214; ADP: MS^3 m/z 426 → m/z 328 → m/z 134; Glucose-1,6-bisphosphate: MS^3 m/z 339 → m/z 241 → [m/z 79 + 97] + m/z 121 + 139; Fructose-1,6-bisphosphate: MS^3 m/z 339 → m/z 241 → [m/z 79 + 97] + m/z 151. ATP and ADP were imaged together on select sections, and glucose bisphosphate and fructose bisphosphate were imaged together on select sections. These transitions were optimized on standard compounds. The transitions for ATP and ADP are selective enough to distinguish them from exact isomers (dGTP and dGDP). The