Glucose transport and neurochemical profile in the hippocampus of STZ-induced diabetic rats under hyper- and euglycaemia studied by in vivo 1H MRS at 9.4T

J. M. Duarte[1,2], R. A. Carvalho[1], R. A. Cunha[1], and R. Gruetter[2]

[1]Center for Neuroscience and Cell Biology, Coimbra, Coimbra, Portugal, [2]Laboratory for functional and metabolic imaging, Ecole Polytechnique Federale de Lausanne, Lausanne, Lausanne, Switzerland

Introduction

Recent epidemiological studies revealed that diabetes mellitus accentuates the age-dependent decline of cognitive function [1] which is related to reduction of hippocampal volume [2], a brain structure involved in learning and memory processing. Streptozotozin can be used to experimental type 1 diabetes in rats, characterized by chronic hyperglycaemia, deficits in learning and memory and impaired hippocampal function [3], as in human diabetes. The aim of the study was to measure the neurochemical profile and the kinetics of glucose transport in the hippocampus of streptozotocin (STZ)-induced diabetic rats using non-invasive ^{1}H NMR spectroscopy.

Materials and Methods

Animal preparation: Type 1 diabetes was induced in Sprague-Dawley rats (8 weeks old) by *i.p.* injection of STZ (65 mg/kg, prepared in sodium citrate buffer 10 mM, pH 4.5), which induced sustained levels of blood glucose above 300 mg/dL after 3 days [4], and maintained for 1 month with food and water *ad libitum*. Age-matched untreated rats were used as controls. The rats were anaesthetized using 2 % isoflurane in oxygen gas for surgery, and then were intubated and ventilated with a pressure-driven ventilator. Catheters were inserted into the femoral artery for monitoring blood gases, glucose and arterial blood pressure, and into the femoral vein for intravenous infusion of α-chloralose, glucose and/or insulin. After surgery the anesthesia was switched to α-chloralose (*i.v.* bolus of 80 mg/kg and infusion of 25 mg $kg^{-1}h^{-1}$). D-glucose [20 % (w/v) solution] was infused at a rate adjustable to the concomitantly measured plasma glucose concentrations to achieve stable target glycemic levels. Insulin (1 U/mL solution) was infused to reduce glycaemia of STZ-treated rats. NMR measurements were performed after each glucose level had been stable for more than 15 minutes. The body temperature was maintained at 37 °C. the arterial PCO2 between 35 and 45 mm Hg and the arterial pH at 7.40.

NMR spectroscopy: All experiments were carried out on an actively-shielded 9.4 T, 31 cm scanner (Varian/Magnex) using a homebuilt 10 mm ^{1}H quadrature surface coil. Shimming was performed with FASTMAP, and ^{1}H NMR spectra were acquired from an 18 µL voxel positioned in the left hippocampus using SPECIAL [5] with TE of 2.8 ms and TR of 4 s. The spectral analysis was carried out using LCModel [6].

Results and Discussion

^{1}H NMR spectra obtained directly showed elevated glucose concentration (at 5.23 ppm) in the hippocampus of the diabetic rat, which returned to control levels upon normalization of glycaemia (Fig. 1), consistent with unaltered hippocampal glucose transport compared to controls (Fig. 2). STZ-treated animals displayed signficantly altered neurochemical profile in the hippocampus (Fig. 2), *e.g.* increased glutamate (Glu), *myo*-inositol (Ins), glycerophosphocholine (GPC), phosphocholine (PCho), taurine (Tau), β-hidroxybutyrate (bHB), *scyllo*-inositol (Scyllo) and total creatine (Cr+PCr) and decreased glutathione (GSH) and *N*-acetylaspartylglutamate (NAAG) concentrations. Glycaemic normalization restored all but the concentrations of *myo*-Inositol and phosphocholine, suggesting that the main effect of diabetes on brain metabolites is due to osmolarity dysregulation that is counterbalanced namely by increased *myo*-inositol, taurine and total creatine. We conclude that (a) chronic hyperglycemia does not alter brain glucose transport/content and (b) glycemia restoration immediately normalizes most neurochemical alterations.

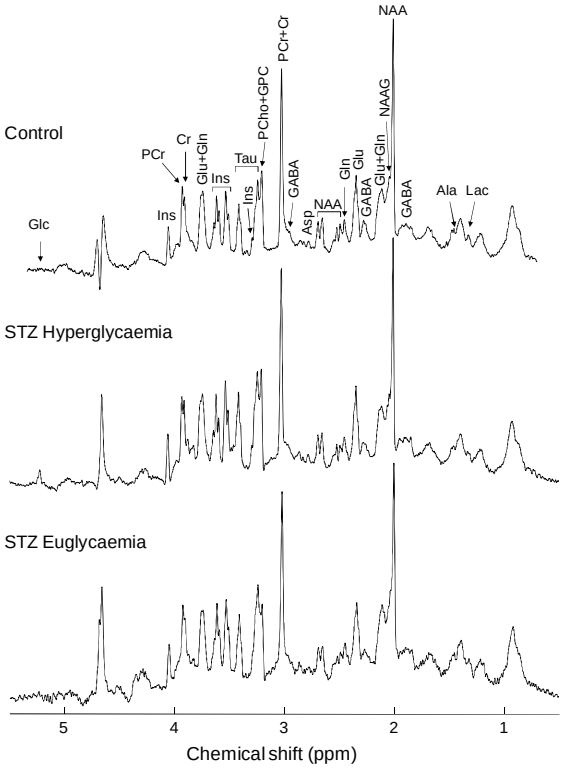

Fig. 1. *in vivo* ^{1}H NMR spectra of the rat hippocampus at 9.4 T, from Control (top) and STZ-treated rats before (center) and after (bottom) glycaemic normalization with insulin infusion.

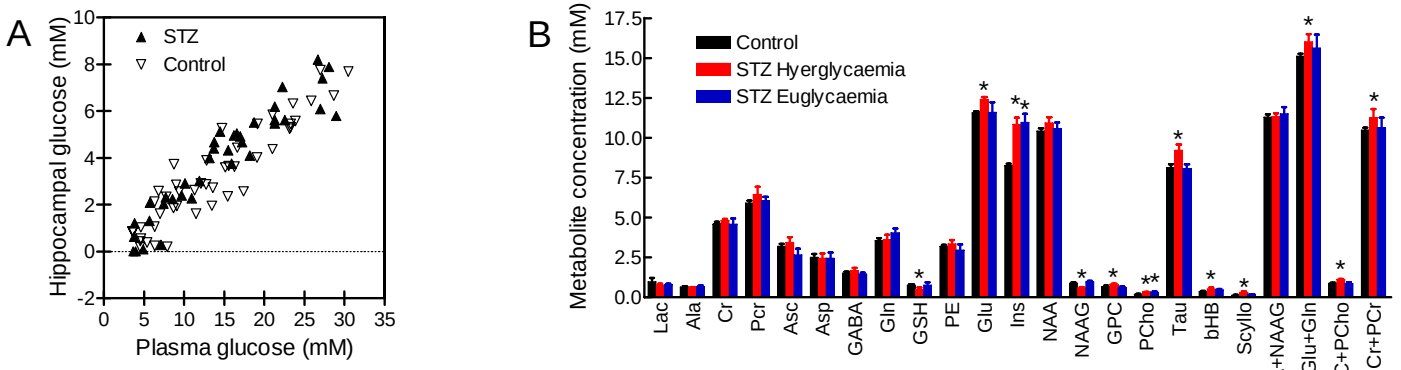

Fig. 2. Hippocampal glucose concentration as function of plasma glucose (left panel) and metabolic profile (right panel) in control (n=6) and STZ-treated rats (n=4) under hyperglycaemia and after glycaemic normalization. From these three experimental groups, glycaemia was 4.9±0.5 mM, 37.6±5.9 mM and 6.2±1.2 mM, respectively. Results are presented as mean±SEM and were analyzed with the Student's t test (* P<0.05).

References: [1] Biessels et al, Lancet Neurol 5:64-74 (2006). [2] Convit et al, PNAS 100:2019-2022 (2003). [3] Biessels et al, Brain Res 800:125-135 (1998). [4] Duarte et al, Neurochem Int 48:144-150 (2006). [5] Mlynárik et al, Mag Reson Med 56:965-970 (2006). [6] Provencher, Mag Reson Med 30:672-679 (1993).

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