Effect of long-term caffeine consumption on glucose transport and osmolarity alterations in the hippocampus of STZ-induced and Goto-Kakizaki diabetic rats: *in vivo* ^{1}H MRS study at 9.4 T

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Introduction:

Diabetes *mellitus* may affect the morphology and plasticity of the hippocampus, leading to cognitive impairment [1; 2]. Our previous work presented to this society [3] showed that streptozotocin (STZ)-induced diabetic rats had similar glucose transport kinetics and, accordingly, displayed osmolarity-related metabolic alterations in the hippocampus. Since chronic STZ-induced diabetes leads to a sustained up-regulation of facilitatory adenosine A_{2A} receptors in the hippocampus [4], blockade of hippocampal A_{2A} receptors may constitute a strategy for neuroprotection and prevention of cognitive deficits upon diabetes. Caffeine is an antagonist of adenosine receptors [5], and we now tested if chronic caffeine intake could prevent metabolic alterations in the hippocampus of STZ-induced and Goto-Kakizaki (GK) diabetic rats, which model insulin-dependent and insulin-resistant diabetes, respectively.

Methods:

Type 1 diabetes was induced in Sprague-Dawley rats (8 weeks old) by i.p. injection of 65 mg/kg of STZ (in sodium citrate buffer 10 mM, pH 4.5), which induced sustained hyperglycaemia after 3 days [4]; rats were maintained with food and water *ad libitum* for 1 month. Age-matched untreated rats were used as controls. Caffeine was provided in the drinking water (1 g/L) starting from 6 weeks old. Due to polydipsia, STZ-treated rats received caffeine solution with concentration adjusted to the water intake. Spontaneously insulin-resistant GK rats and Wistar control rats received caffeine from 2 to 6 months of age. Body weight, glycaemia, insulinemia, caffeine intake and serum caffeine were controlled. The NMR experiment was performed at 1 month of diabetes for STZ-treated rats and at 6 months of age for GK rats (as well as for the respective controls).

Rats were anaesthetized with 2% isoflurane in oxygen, and then intubated and ventilated with a pressure-driven ventilator. Catheters were placed into femoral artery for monitoring blood gases, glucose and blood pressure, and into femoral vein for infusion of α-chloralose, glucose and/or insulin. Anaesthesia was then switched to α-chloralose (i.v. bolus of 80 mg/kg and infusion of 25 mg $kg^{-1}h^{-1}$). Glucose [20% (w/v) solution] was infused at a rate adjustable to the concomitantly measured plasma glucose concentrations to maintain the desidered glycaemia 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. Body temperature was maintained at 37 °C, arterial PCO_2 between 35 and 45 mm Hg, and arterial pH at 7.40.

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 [6] with TE of 2.8 ms and TR of 4 s. The spectral analysis was carried out using LCModel [7].

Results and Discussion:

As previously demonstrated for STZ-treated rats [3], GK diabetic rats also displayed unaltered glucose transport kinetics into the hippocampus (not shown). Long-term caffeine intake failed to affect glucose transport into the hippocampus in both models of diabetes (not shown). The high glucose concentration in the diabetic hippocampus triggered metabolic alterations related to the osmotic adaptation, in rats either consuming caffeine or not. The total osmolyte concentration [*myo*-inositol + taurine] was not modified by caffeine intake in the diabetic hippocampus (figure 1). However, in the hippocampus of STZ-treated rats, caffeine altered the relative levels of taurine and *myo*-inositol, being taurine levels higher when caffeine was consumed. In the hippocampus of GK rats, taurine but not *myo*-inositol was used to regulate osmolarity. We conclude that *myo*-inositol and taurine are used as osmolytes to counterbalance high hippocampal glucose concentration, and their relative contribution is dependent on the type or severity of the diabetic condition. Due to the anti-oxidant properties of taurine [8], the caffeine-induced increase in the level of taurine rather than *myo*-inositol can have a neuroprotective role, and simultaneously maintain the osmotic adaptation of the diabetic hippocampus to sustained high glucose levels upon uncontrolled diabetes.

References: [1] Brayne *et al.* (2005) Neurobiol Aging 26 Suppl 1, 6. [2] Convit A (2005) Neurobiol Aging 26 Suppl 1, 31. [3] Duarte *et al.* (2008) Proc Intl Soc Mag Reson Med 16, 2102. [4] Duarte *et al.* (2006) Neurochem Int 48, 144. [5] Fredholm *et al.* (1999) Pharmacol Rev 51, 83. [6] Mlynárik *et al.* (2006) Mag Reson Med 56, 965. [7] Provencher (1993) Mag Reson Med 30:672. [8] Di Leo et al. (2004) Amino acids 27, 187.

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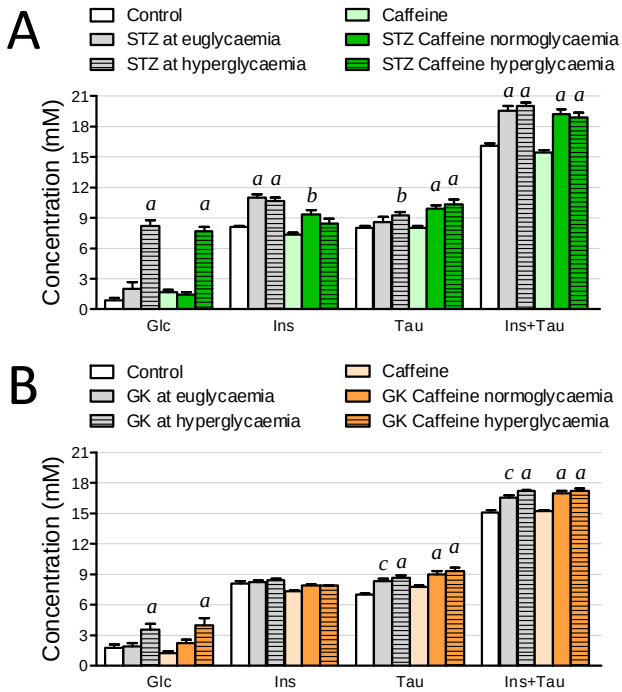

Figure 1. Glucose (Glc), *myo*-inositol (Ins) and taurine (Tau) concentrations in the hippocampus of STZ-induced and GK diabetic rats under hyper- or normoglycaemia, treated or not with caffeine. Data are presented as mean±SEM, and were compared with two-way ANOVA followed by Bonferroni's test (*a* P<0.001, *b* P<0.05, *c* P<0.01, compared to controls).