Metabolic studies of glucose transporter 2 knockout (RIPGLUT1GLUT2-/-) mice using in vivo ^{1}H MRS and CASL

Hongxia Lei[1,2], Frederic Preitner[3], Bernard Thorens[3], and Rolf Gruetter[1,3]

[1]*Ecole Polytechnique Fédérale de Lausanne, Lausanne, Vaud, Switzerland,* [2]*University of Geneva, Geneva, Geneva, Switzerland,* [3]*University of Lausanne, Lausanne, Vaud, Switzerland*

INTRODUCTION

The functionality of glucose transporter isoform 2 (GLUT2) in brain remained not well understood. GLUT2 was mainly located in neurons and could involve in brain specific glucose sensing mechanism, such as being insensitive to hyperglycemia or hypoglycemia or both (1). Deletion of GLUT2 (GLUT2$^{-/-}$) might influent hormonal responses to regulate glucose homeostasis, for example, increasing cerebral blood flow (CBF) at acute hypoglycemia. Therefore, studying mouse models will intrinsically provide important insights in understanding the underneath mechanism and its pathogenesis. Recently, ^{1}H MRS and CASL studies at high magnetic fields became feasible in mouse, for instance, hippocampus and hypothalamus (2,3). Therefore, the aim of this study was to apply both localized ^{1}H MRS and CASL on GLUT2$^{-/-}$ mice and their counter WT types at 9.4T to study the effects of GLUT2$^{-/-}$ on brain, in particular hippocampus and hypothalamus.

METHODS

Animals: GLUT2$^{-/-}$ mice were derived along with transgenic re-expression of GLUT1 in the pancreatic β cells as previously described (RIPGLUT1GLUT2$^{-/-}$, 1). All animal experiments were with the approval by the local ethic committee. At age of 16-20th weeks, animals (n=19) were immobilized under 3% isoflurance mixed with air and oxygen (2:1) and well maintained under 1-2% isoflurance thereafter to maintain breathing rates >100bpm. Such rates had been shown capable of maintaining mice under physiological conditions, such as PCO_2 in the range of 35-45mmHg (preliminary data not shown). Tail bleeds were sampled and measured for glucose levels (Breeze glucose meter) immediately before and right after MR measurements, which were approximately one and a half hours.

MR measurements: All MR measurements were performed at 9.4T (26cm diameter). Localized ^{1}H MRS spectroscopy was applied in hippocampus and hypothalamus as previously described (2). 320-480 scans were acquired to sustain sufficient SNRs. CBF was measured using a well-established CASL technique in combination with a home-built actively-detuned system (3). Four segmented semi-adiabatic EPI sequence was adopted with a labeling module to implement the CASL sequence (3 consecutive 2mm-thick slices, 23×15mm^2, 128×64 data matrix).

Data Analysis: CBF maps were calculated from 16 paired labeled and controlled images with a labeling efficiency 0.8 (3). MR spectra data were processed and analyzed as previously by referencing to unsuppressed water signal (2). Significant difference was when $p<0.05$.

Table1 Summary of CASL on regional blood flow

Region	WT (ml/100g/min)	KO (ml/100g/min)
hippocampus	111.7±14.0	122.4±7.4*
hypothalamus	91.3±9.9	99.0±16.1
cortex	119.7±15.6	123.9±11.9
brain	102.7±6.3	106.4±6.6

"*" indicated p=0.0002 using unpaired student t-test.

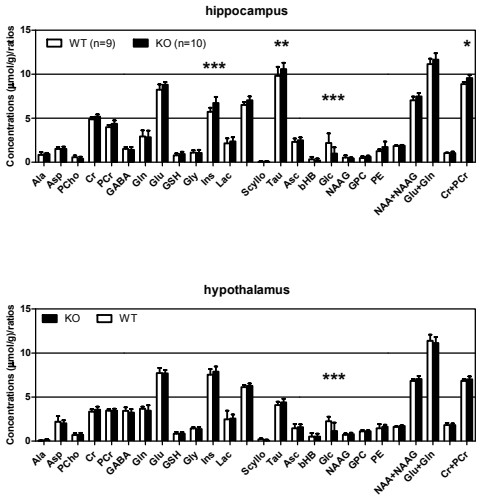

Figure 1. Metabolic profiles of hippocampus and hypothalamus of RIPGLUT1GLUT2$^{-/-}$ mice (KO, solid black bars, n=10) and their counter WT types (WT, solid white bars, n=9). "*" indicated $p<0.05$ and "***" represented $p<0.001$ when using two-way ANOVA.

RESULTS AND DISCUSSION

Right before the animals were ready for the MR measurements, the blood glucose levels in RIPGLUT1GLUT2$^{-/-}$ mice were 7.1±2.7mM, slightly lower that in their WT countertypes, i.e. 10.5±1.5mM. Immediately after the MR measurements, the blood glucose levels of RIPGLUT1GLUT2$^{-/-}$ mice reduced to 5.4±1.7mM, which became significantly different when compared to those in the WT mice, 10.0±5.2mM (p=0.0006, student paired t-test). While at euglycemia, CBF was globally higher in RIPGLUT1GLUT2$^{-/-}$ mice and significant in hippocampus (Table 1). The consistently increased blood flow has been observed here was similar to rat brain under insulin induced hypoglycemia (4). Other than regional CBFs, taurine, myo-inositol and total creatine, were increased in hippocampus of RIPGLUT1GLUT2$^{-/-}$ mice when using localized ^{1}H MRS, as listed in Figure 1. The observations at euglycemia suggested that GLUT2 indeed affected brain tissue, i.e. hippocampus, substantially. On top of that, it is interested to note that except brain glucose, the elevation of myo-inositol (Ins) and taurine (Tau) in RIPGLUT1GLUT2$^{-/-}$ mice were similar as previous observations in STZ induced diabetic animal brains, which were known to maintain water balance of brain at chronic hyperglycemic or diabetic conditions (5). Nonetheless, the absence of GLUT2 did alter osmoregulation in brain possibly through other mechanism. We concluded that GLUT2 played an important role in glucose sensing mechanism in brain and the alternation of metabolites in hippocampus indicated a different adaption mechanism other than osmoregulation observed in diabetic animals.

REFERENCES 1. Thorens B et al. 2000 J Biol Chem 275:23751; 2. Lei H et al. 2010 NMR Biomed 23(6):578; 3. Lei et al. 2011 Conf Proc IEEE EMBS 2011, In Process; 4. Behar KL 1995 J Neurochem 44(4):1045; 5. Duarte JM et al. 2009 J Neurochem 111(2):368

ACKNOWLEDGMENTS Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL,UNIGE, HUG, CHUV, EPFL, SNF 3100A0-122498, and the Leenaards and Jeantet Foundations.