Cerebral blood flow of mouse increases upon insulin-induced hypoglycemia using the continuous arterial spin labeling technique

Hongxia Lei[1,2] and Rolf Gruetter[1,3]
[1]*University of Geneva, Geneva, Geneva, Switzerland,* [2]*EPFL, Lausanne, Vaud, Switzerland,* [3]*University of Lausanne, Lausanne, Vaud, Switzerland*

INTRODUCTION

Non-invasively studying cerebral metabolic responses upon hypoglycemia is of great interest to clinical practice on diabetic patients. For instance, how brain responses to hypoglycemia conditions might cause irreversible damages and could play an important role in hypoglycemia unawareness. Therefore, investigating transgenic animals would help obtaining insightful information on hypoglycemia-induced metabolic responses, potential damages and consequences thereafter in brain. MR is non-invasive and applicable to human. In addition, MR provides multi-modalities for numerous objectives, such as anatomy, diffusion, perfusion, function and metabolites etc. Among these MR modalities, perfusion MRI in mice upon hypoglycemia remains difficult mainly due to reduced sample size and technical challenges in MR instrumentation. Recently, the arterial spin labeling (ASL) technique at high magnetic fields was feasible in mice, such as cardiac ASL (cASL, 1), continuous ASL (CASL, 2) and pseudo-continuous CASL (pCASL, 3) etc. Among these ASL methods, the CASL technique using an actively-detuned system has been shown to supply the highest labeling efficiency, i.e. 0.82 (2). Therefore, we aimed to evaluate feasibility in detection of hypoglycemia induced blood flow changes in mice using CASL.

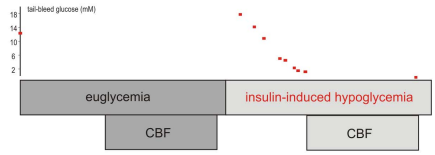

Figure 1. A schematic layout of our experimental design along with a plot of glycemic levels in one mouse.

METHODS

Animals: All experiments were performed with the approval by the local ethic committee. At age of 16-20th weeks, six C57/BL male mice were immobilized under isoflurane with a percentage at 3% mixed with air and the percentage of isoflurane was thereafter maintained in the range from 0.8 to 2% to sustain breathing rates above 100 beats-per-minute. Such rates had been shown capable of maintaining mice under physiological conditions, such as $PaCO_2$ in the range of 35-45mmHg (1). Tail bleeds were sampled and measured for glucose levels (Breeze glucose meter).

MR measurements: All MR measurements were performed at 9.4T (26cm-diameter). Once field homogeneity was optimized, CBF was measured using the CASL technique in combination with a home-built actively-detuned system (2). Four segmented semi-adiabatic EPI sequence was adopted with a labeling module to implement the CASL sequence (3×2mm-thick slices with a 0.2mm gap, 23×15mm^2, 128×64 data matrix). CBF maps were calculated from 16 paired labeled and controlled images with a labeling efficiency 0.8 (2). Animals were measured under euglycemia and hypoglycemia. The percent changes were calculated. Significant difference was when $p<0.05$.

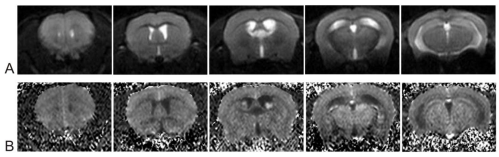

Figure 2. Typical 4-segemented semi-adiabatic SE-EPI images (A) and the calculated CBF maps (B) at 9.4T.

Hypoglycemia: An adjusted insulin infusion protocol, i.e. a bolus followed by a continuous rate, induced hypoglycemia in mice. Animals reached hypoglycemia conditions when glucose levels were less than 2.5mM. The hypoglycemic clamps were performed outside the magnet while animals remained in the holder. The entire experimental protocol was illustrated in Figure 1.

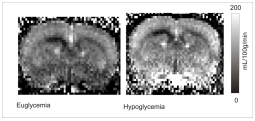

Figure 3. CBF maps of one mouse under Euglycemia and hypoglycemia. A clear elevation in CBF was observed.

RESULTS AND DISCUSSION

The tail-bleed glucose levels before insulin infusion were 9.8±1.5mM (mean±SEMs), in the range of 4.7-13.3mM, which were beyond hypoglycemia. When administrating 25.2±5.9UI/kg insulin, hypoglycemia was reached to 1.6±0.2mM (mean±SEMs), in the range of 1.2-2.3mM. With such experimental design (Figure 1), SE-EPI images can be obtained with minimal distortion and well-defined anatomical structure (Figure 2A) before and during hypoglycemia. The calculated CBF maps (Figure 2B) were in satisfactory quality to deliver regional blood flow. During hypoglycemia, elevated blood flow was observed over the entire brain (Figure 3). The quantified results suggested that the percent changes of cerebral blood flow upon hypoglycemia were within 50% and significant when comparing to euglycemic conditions (Figure 4). This is consistent with previous rat studies but slightly lower than those in rats (4). The difference here could be explained partially by isoflurane anesthesia in mice, unlike apha-chloralose anesthesia in rats (4). Overall, this will not change our main conclusion that the CASL technique can be applied to study cerebral blood flow responses upon hypoglycemia in mice. This opens possibility in studying transgenic or diabetic mouse models by means of multiple MR modalities.

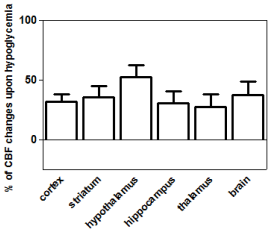

Figure 4. Percent of CBF changes upon hypoglycemia in six mice. Error bars are SEMs.

REFERENCES 1. Nair G et al. MRM 2008; 2. Lei H et al. 2011 Conf Proc IEEE EMBS 2011 ; 3. Duhamel G et al. ISMRM 2011; 4. Choi IY et al. JCBFM. 2001

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