In vivo ^{1}H MR studies of cortical metabolic response during insulin-induced hypoglycemia

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INTRODUCTION

Hypoglycemia induced energy deficits have been intensively studied through both in *vitro* and *in vivo* approaches due to received interests in clinical treatment of diabetes. Cerebral blood flow (CBF) and brain energy metabolites have been suggested to be involved in hypoglycemia (1-3 and references therein). Thus simultaneously studying both CBF and neurochemical metabolites can help understanding of the underlying mechanism of hypoglycemia induced energy deficits. MR techniques allow assessment of energy status (4), neurochemical constituents (4,5) and functional status (2) etc. With improved MR hardware and techniques, ^{1}H MRS of regional brain tissue, such as cortical tissue can be studied in rats (6). Therefore, we explored the feasibility to study hypoglycemia in a step-down function using localized ^{1}H MRS for neurochemical metabolites and continuous arterial spin labeling (CASL) for CBF.

METHODS

Under local veterinary authorities, seven male SD rats (270-350g) were prepared for MR studies under 2% isoflurane and switched to light α-chloralose anesthesia (~27mg/kg/hr continuous rate) immediately afterwards (2,6). Four stages of glycemia, mild hyperglycemia (<15mM), mild hypoglycemia (2-3.5mM), hypoglycemia (1-2mM), severe hypoglycemia (<1mM) and mild hyperglycemia recovery (>5mM) were reached by continuously infusing both glucose (20% w/v) and insulin (2UI/mL). All animals were monitored for breathing, temperature and blood-pressure (90-200mmHg) using a MR compatible monitor system (SA Instruments) and rectal temperature was maintained at 37°C by circulating warm water. Blood gases were maintained within a normal physiological range (pH~7.4, $PaCO_2$~35-50mmHg, PaO_2>90mmHg) throughout the entire studies based on the concomitant arterial blood measurement using a nearby analyzer. In addition, plasma glucose was measured using a nearby glucose analyzer and well-maintained at steady-state levels by adjusting infusion rates accordingly. All MR studies were performed at 9.4T. Localized ^{1}H MRS was applied on cortex (~40µL) using SPECIAL (TE/TR=2.8/4000ms, NT=160-320). MR spectra were processed and quantified using LCModel (5 and references therein). For CBF, two-coil system (one 0.8-mm-inner-diameter butterfly coil and one quadrature coil with two physically decoupled 14-mm-inner diameter loops) was adopted for CASL as previously (2). These two coils were well decoupled (<-30dB) and thus led to minimal magnetization transfer effects. 32 pairs of 2-mm slice thickness, single-shot GRE-EPI coronal images (RO×PE= 25×25mm^2, 64×64 data matrix) at bregma 0-mm were acquired with a labeling module (gradient (1G/cm) and RF pulse (3sec, amplitude modulated)) or with no additional labeling module. An additional 6 sec delay resulted in 10min for this acquisition. CBF was calculated from cortex as previously described (2). The magnetization transfer effects were evaluated on animals sacrificed at the end of studies.

RESULTS AND DISCUSSION

Five of animals underwent severe hypoglycemia and four of them recovered afterwards within physiological ranges. Throughout the entire studies, noticeable changes in both MR spectra and CBF images appeared at severe hypoglycemia (Figure 1C). Further quantitative results of cortical blood flow at euglycemia (42.3±3.4ml/100g/min, 6.2±2.1mM) was nearly unaltered at mild hypoglycemia (42.4±4ml/100g/min, 2.6±0.4mM), but increased by ~50% to 60.4±7.9ml/100g/min at hypoglycemia (1.2±0.2mM, $p<0.01$, Figure 2), and further raised to an elevated level at severe hypoglycemia (0.6±0.1mM), i.e. 190.1±35.0ml/100g/min ($p<0.0001$, Figure 2). When glycemia (9.3±1.3mM) was restored, cortical blood flow returned to normal levels, i.e. 51.0±11.0ml/100g/min. Concurrently, ^{1}H MRS measurements of cortical tissues resulted in substantial metabolic information and confirmed the spectral observation in figure 1 (Figure 3). Significant reductions in Glu, Gln PCr/Cr along with increased Asp were observed only in severe hypoglycemia (Figure 1 and 3). The metabolic changes were consistent with previous *in vitro* results along with hypoglycemia coma (iso-electric conditions, 4, 7), which indicated strongly coupled neurochemical alterations in cortex and CBF responses in rat cortical tissue to insulin-induced hypoglycemia. In conclusion, ^{1}H MR can allow measuring valuable neurochemical constituent information in conjunction with blood flow response during insulin-induced hypoglycemia. This opens possibilities of studying the mechanism of hypoglycemia and help consolidating *in vivo* observations and electrical physiology observations.

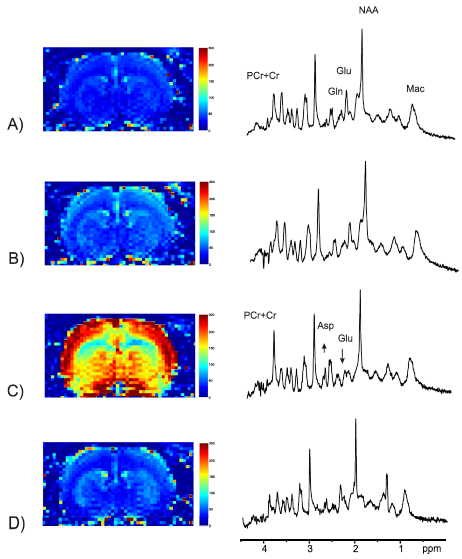

Figure 1: CBF images (1st column) and ^{1}H MR cortical spectra (2nd column) of one rat before, during and after insulin-induced hypoglycemia at plasma glucose levels of 4.5mM(A), 1.3 mM(B), 0.6mM (C) and 11mM (D). CBF images were displayed in the range of 0-300mL/100g/min. MR spectra were acquired (TE/TR=2.8/4000ms, NT=320) and displayed with apodization (gf=0.15sec). Selected major metabolites were assigned. Metabolic changes were marked in (C), such as increased Asp and decreased Glu. Abbreviations: Asp, aspartate; Cr, creatine; Gln, glutamine; Glu, glutamate; PCr, phosphylcreatine; Mac, macromolecule; NAA, N-acetylaspartate.

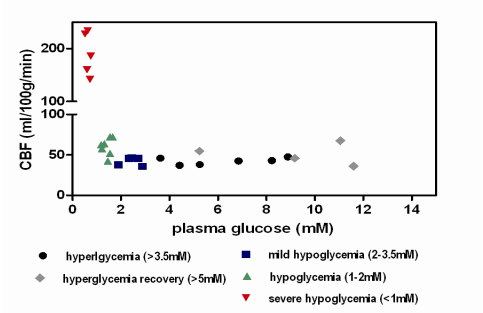

Figure 2: Quantitative cortical blood flow over five glycemic statuses.

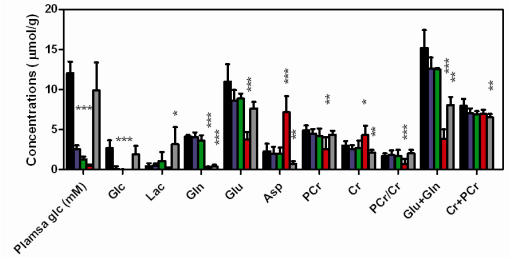

Figure 3: Summary of MR spectral results along with plasma glucose levels over all experiments, mild hyperglycemia(black), mild hypoglycemia (blue), hypoglycemia(green), severe hypoglycemia (red) and hyperglycemic recovery (gray). When plasma glucose and brain glucose were significant lower than those at euglycemic levels using student paired t-test, significant changes in metabolites were observed and indicated with significance levels: "*" as $p<0.01$, "**" as $p<0.001$ and "***" as $p<0.0001$. Abbreviations: Lac, lactate; Glc, glucose.

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