Neurochemical changes in hippocampus of developing rats during acute hypoglycemia

I. Tkac[1], K. Ennis[2], and R. Rao[2]

[1]Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, United States, [2]Department of Pediatrics, University of Minnesota, Minneapolis, MN, United States

INTRODUCTION Hypoglycemia is a common metabolic condition in newborn infants. Prolonged and recurrent hypoglycemia is associated with long-term hippocampal dysfunction [1,2]. Despite extensive studies in humans and animal models, detailed mechanism is not well understood [3,4], especially the sequel of different processes providing fuel for brain cells, when glucose plasma is too low. The aim of this study was measure neurochemical changes during acute hypoglycemia in the hippocampus of rat pups using ^{1}H NMR spectroscopy at 9.4 T.

METHODS All NMR measurements were performed using a Varian INOVA spectrometer interfaced to a 9.4 T magnet, equipped with powerful gradient/shim coils insert (Resonance Research Inc). First and second order shims were adjusted by FASTMAP [5]. Ultra-short echo-time STEAM (TE = 2 ms) combined with outer volume suppression and VAPOR water suppression was used for ^{1}H NMR spectroscopy [6]. Metabolite concentrations were quantified using LCModel with macromolecule spectra included in the database and the unsuppressed water signal was used as an internal reference [7]. Hypoglycemia was induced by insulin (6 IU/kg, i.p.) in 14-day-old Sprague-Dowley rats pups (n=6), followed by rescue 240 min later using 10% dextrose. Saline was given to control rats (n=2). Rats were anesthetized with isoflurane, actively ventilated and the ECG, $ETCO_2$ and body temperature were continuously monitored.

RESULTS AND DISCUSSION After insulin administration plasma Glc dropped below the "hypoglycemia threshold" (2.5 mM) within 60 min and stayed at 1.5 ± 0.8 mM for the next 180 min. Of all 16 metabolites simultaneously measured during hypoglycemia, only aspartate (Asp), creatine (Cr), glucose (Glc), glutamine (Gln), glutamate (Glu), lactate (Lac) and phosphocreatine (PCr) exhibited significant changes (Fig. 1 and 2). Within the first 120 min brain Glc decreased below the detection threshold (0.5 µmol/g), Lac decreased by 50%, however, the PCr/Cr ratio remained at normal physiological value. During this period, the decrease in the spectral linewidths (from 9 to 7 Hz) was always observed, which resulted from the positive BOLD effect caused by the increased CBF. During the second stage (120 – 160 min), Gln and Glu started to decrease with the simultaneous increase of Asp. During the subsequent very short period (160 – 180 min), dramatic changes in the neurochemical profile occurred in the hippocampus. PCr/Cr ratio and Gln decreased nearly to zero, Glu decreased below 25% and Asp increased up to 300% of their original values. During the fourth phase (180 – 230 min) Glc, Gln, Glu and PCr remained extremely low and Asp gradually decreased. After 180 min of hypoglycemia, rat pups were rescued by the i.p. bolus of dextrose, which resulted in fast recovery of Glu and PCr/Cr, but not Gln close to physiological values. Very similar time courses were observed in all 6 studied animals and no change was observed for any metabolite in control experiments. Very minor changes in the first period indicate that another endogenous substrate, most likely glycogen, was used to compensate insufficient supply of Glc from the blood despite increased CBF. Once Glc and Lac significantly decreased and glycogen stores were exhausted anaplerotic processes became dominant to replenish TCA cycle intermediates. The decrease of Glu and the increase in Asp clearly demonstrate the production of α-ketoglutatate from Glu via transamination reaction. The decrease of Glu by 6 µmol and Gln by 2 µmol was accompanied only by 4 µmol increase of Asp, which indicates that other anaplerotic processes, such as direct conversion of Glu to α-ketoglutatate catalyzed by glutamate dehydrogenase, significantly contributed to fuel the TCA cycle. When Glu decreased below 2 µmol/g, Asp became the last source of carbon for the TCA cycle, probably with involvement of malate dehydrogenase reaction to replenish pyruvate. In conclusion, these results clearly demonstrate that prolonged hypoglycemia results in enormous deviation in hippocampal homeostasis. When endogenous Glc resources were exhausted, amino acids fed TCA cycle through different anaplerotic pathways. The duration of hypoglycemia is critical to avoid irreversible brain damage.

REFERENCES: 1. Lucas A et al.,*BMJ* 1988: 297,1304-1308; **2.** McNay EC et al., *Diabetes* 2006: 55, 1088-1095; **3.** Isaev NK et al., *Biochem (Moscow)* 2007: 72, 471-478; **4.** Yamada KA et al., *Pediatr Res* 2004: 55, 372-379; **5.** Gruetter R and Tkac I, *Magn Reson Med* 2000: 43, 319-323; **6.** Tkac I et al., *Magn Reson Med* 1999: 41, 649-656; **7.** Pfeuffer J et al., *J Magn Reson* 1999: 141, 104-120.

Supported by: *Keck Foundation, Viking Children's Fund, Graduate School of Univ. of MN and NIH grants P41-008079, P30 NS057091 and HD47276*

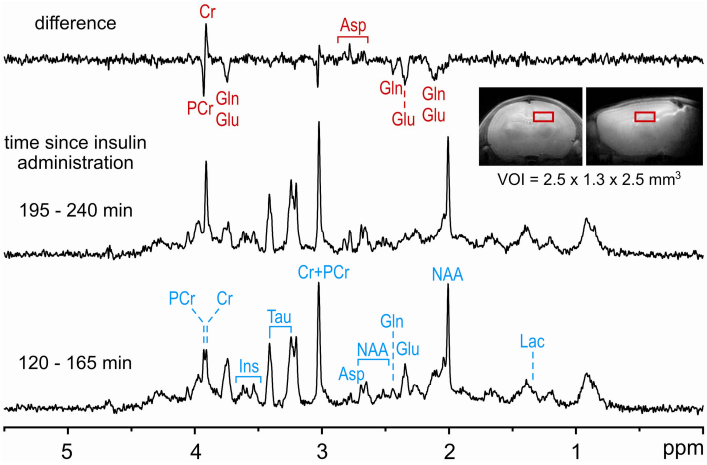

Fig. 1 In vivo ^{1}H NMR spectra from hippocampus of P14 rat during different time period of hypoglycemia. Top trace: difference spectrum

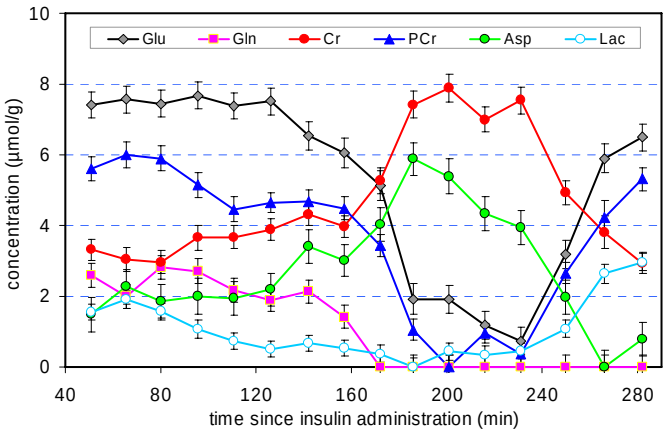

Fig. 2 Time courses of metabolites in hippocampus of P14 rat during insulin induced hypoglycemia.