Comparison of metabolic fluxes in the mouse dorsal hippocampus and hypothalamus using indirect ^{1}H-[^{13}C] MRS upon [1,6-$^{13}C_2$] glucose infusion

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Synopsis

With increased interests in understanding the hippocampal regulation of hypothalamic-pituitary-adrenocortical (HPA) axis, this study shows for the first time an *in vivo* comparison of metabolic fluxes in the mouse dorsal hippocampus and hypothalamus using indirect ^{1}H-[^{13}C] MRS upon [1,6-$^{13}C_2$] glucose infusion at 14.1T. This study provides foundation for investigating relevant evidence to the underlying mechanism of hippocampal regulation of HPA axis.

INTRODUCTION

The hippocampus plays a critical role in linking brain energetics and behavior typically observed in the response to stress. With increased interests in the hippocampus as a potential regulator of the hypothalamic-pituitary-adrenocortical (HPA) axis, the final common pathway in the stress response,[1] differentiating glucose metabolism between dorsal hippocampus and hypothalamus may lay foundation for investigating potential mechanisms of HPA sensitivity regulation by the hippocampus.
^{13}C Magnetic Resonance Spectroscopy (MRS) has shown to be a very promising tool to study brain metabolism in vivo. Indirect carbon spectroscopy (^{1}H-[^{13}C] MRS) is a technique that allows measuring ^{13}C-labelled metabolites with higher sensitivity.[2] Recently, metabolic fluxes including TCA cycle in mouse hypothalamus were assessable using ^{1}H-[^{13}C] MRS upon infusion of [1,6-$^{13}C_2$] glucose at 14.1T.[3]
Thus we aimed to compare glucose metabolism of the mouse dorsal hippocampus and hypothalamus upon [1,6-$^{13}C_2$] glucose.

METHODS

Fourteen adult male C57BL/6 mice (25±2g) were scanned under isoflurane anesthesia (1-2%) in a horizontal 14.1T/26cm Varian magnet (Agilent Inc., USA). A homemade ^{1}H surface coil in quadrature combined with a linear ^{13}C coil was designed specifically for ^{1}H-[^{13}C] MRS. The bilateral dorsal hippocampus (2×6×1.5 mm^3) and hypothalamus (2×2.7×2.2 mm^3) were localized using a set of T_2-weighted FSE images. Field inhomogeneity was adjusted using FASTMAP to reach a water linewidth <25Hz[1]. On the target VOI, localized indirect ^{1}H-[^{13}C] detection was applied using the full signal intensity BISEP-SPECIAL sequence (TE/TR=2.8/4000ms) together with OVS and water suppression.[2,3] Glucose metabolism of both bilateral dorsal hippocampus (n=7) and hypothalamus (n=7) was evaluated upon a bolus of 99% enriched 20% (w/v) [1,6-$^{13}C_2$] glucose and followed by continuous infusion of 70% enriched 20% (w/v) [1,6-$^{13}C_2$] glucose up to 4 hours. Spectra were frequency corrected and summed (~5.5min of hippocampus and ~11min for hypothalamus) for LCModel quantification. Non-edited ^{1}H MR spectra contain ^{1}H resonances coupled to both ^{12}C and ^{13}C and thus can be quantified with a standard basis set for neurochemical profiles of mouse hippocampus.[2,3] The ^{13}C-editing spectra were quantified using another simulated basis set as previously.[2,3] The fraction of isotope enrichment (FE) in lactate (Lac, LacC3), glutamate (Glu, GluC4), glutamine (Gln, GlnC4), the sum of Glu and Gln (Glx, GlxC3) and γ-aminobutyric acid (GABA, GABAC2 and GABAC3) was obtained. The FEs of plasma glucose (Glc), Lac and acetate (Ace) were measured on three mice with the identical infusion protocol without any MR measurements. All results were then fitted to a one compartment model of glucose metabolism (**Figure 1**) using MATLAB nonlinear regression methods.[2,3] The cerebral metabolic rate of glucose (CMR_g), tricarboxylic acid cycle (V_{TCA}), a dilution flux from blood lactate (V_{dil}^{in} and V_{dil}^{out}) and from blood acetate (V_{dil}^{g}), a transmitochondrial flux (V_x), apparent neurotransmission rate (V_{NT}), pyruvate carboxylase flux (V_{PC}), a Gln efflux (V_{eff}), GABA flux (V_{GABA}), and exchange between two GABA pools and two Gln pools (V_{ex}^{g} and V_{ex}^{i}) were estimated. Monte–Carlo simulation was used to evaluate the errors of all adjusted metabolic fluxes.[2,3]

RESULTS AND DISCUSSION

Based on anatomical images with satisfactory quality, bilateral dorsal hippocampus and hypothalamus were clearly identified (**Figure 2**). The typical ^{1}H-[^{13}C] MR non-edited spectra of hippocampus (**Figure 2a**, SNRs=20±2, metabolic linewidths=18±2Hz) and hypothalamus (**Figure 2b**, SNRs=16±3, metabolic linewidths =17±2Hz) were obtained in jointly with edited spectra of both hippocampus (**Figure 2c**, SNRs=8.3±0.6) and hypothalamus (**Figure 2d**, SNRs=5.3±0.3) after 4 hours of [1,6-$^{13}C_2$] glucose infusion, showing clear metabolites labeling. For instance, ^{13}C-coupled ^{1}H resonances of LacC3, GABAC3, GluC3, GlnC3, GABAC2, GluC4, GlnC4, GlxC2, GlcC6 and AspC3 were clearly visible (**Figure 2c and 2d**).
GlcC6 rose rapidly, nearly in a step-wise fashion, shortly reaching a plateau enrichment at 0.66±0.05 (0.69±0.09 in the first half an hour) for hypothalamus and at 0.78±0.06 (0.69±0.01 in the first half an hour) for hippocampus, respectively. In **Figure 3**, LacC3 also rose quickly and reached a plateau around 15min after the bolus, with FEs at 0.51±0.02 for hypothalamus and at 0.52±0.02 for hippocampus, After about 100-130 minutes of glucose infusion, Glu and Gln reached their perspective FE steady-states, i.e. 0.51±0.02 for GluC4 and 0.51±0.01 for GlnC4 in hypothalamus and different from 0.50±0.01 for GluC4 (p=0.004) and 0.46±0.02 for GlnC4 (p<0.0001) in hippocampus. FEs of hippocampal GlxC3 and GlxC2 were 0.42±0.02 and 0.35±0.02, while FEs in hypothalamus were 0.46±0.03 (p=0.001) and 0.34±0.03, respectively. FEs of AspC3 were 0.34±0.06 for hippocampus and 0.38±0.09 for hypothalamus. GABAC2 reached a plateau FE approximately at 150 min, 0.43±0.03 in hypothalamus and 0.42±0.03 in hippocampus. A stable FE of GABAC3 was reached only towards the end of the measurements, i.e. 0.36±0.03 in hypothalamus and 0.38±0.03 in hippocampus, respectively. The averaged FE time courses of GlcC6, LacC3, GluC4, GlnC4, GlxC3 and GABAC2 and GABAC3 were reliably measured and therefore used for modeling (**Figure 1**). The resulting fits of time courses of ^{13}C accumulation (**Figure 3**) are shown in **Figure 4** and the fluxes are summarized in **Table 1**.

This study shows for the first time an in vivo comparison of metabolic fluxes in the mouse dorsal hippocampus and hypothalamus using indirect ^{1}H-[^{13}C] MRS upon [1,6-$^{13}C_2$]-glucose infusion. This study provides foundation for investigating relevant evidence to the underlying mechanism of hippocampal regulation of HPA axis.

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References

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Figures

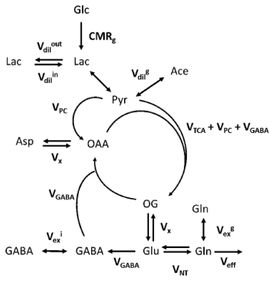

Figure 1. Scheme of the mathematical model used to describe ^{13}C label incorporation into cerebral metabolites during ^{13}C labeled glucose infusion. Abbreviations are the cerebral metabolic rate of glucose (CMR_g), tricarboxylic acid cycle (V_{TCA}), a dilution flux from blood lactate (V_{dil}^{in} and V_{dil}^{out}) and from blood acetate (V_{dil}^{g}), a transmitochondrial flux (V_x), a neurotransmission rate (V_{NT}), pyruvate carboxylase flux (V_{PC}), a Gln efflux (V_{eff}), GABA flux (V_{GABA}), and two GABA pools (V_{ex}^{g} and V_{ex}^{i}).

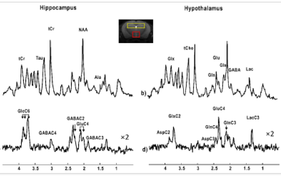

Figure 2. Typical ^{1}H-^{13}C MR non-edited (a, b) and edited (c, d) spectra of one of each mouse hippocampus (yellow voxel, 1Hz Lorentzian apodization) and hypothalamus (red voxel, 2Hz Lorentzian apodization). Abbreviations: Ala, alanine; Asp, aspartate; Gln, glutamine; Glu, glutamate; Glx, glutamine + glutamate; Tau, taurine; tCr, total creatine; GABA, γ-amino-butryrate acid; Lac, lactate; Glc, glucose. Proton resonances bounding to specific carbons are indicated by C and followed by the position number, e.g. C2, C3 and C4 etc.

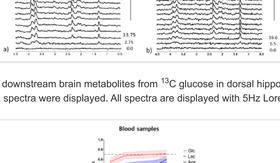

Figure 3. Time courses (in minutes) ^{13}C labelling of downstream brain metabolites from ^{13}C glucose in dorsal hippocampus (a) and hypothalamus (b). In a), every other edited ^{1}H-[^{13}C] MR spectra were displayed. All spectra are displayed with 5Hz Lorentzian apodization.

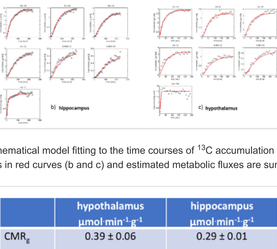

Figure 4. FEs of blood Glc, Lac and Ace (a) and mathematical model fitting to the time courses of ^{13}C accumulation in both hypothalamus (b) hippocampus (c). The fitting results are shown as in red curves (b and c) and estimated metabolic fluxes are summarized in Table 1.

	hypothalamus µmol min^{-1} g^{-1}	hippocampus µmol min^{-1} g^{-1}
CMR_g	0.39 ± 0.06	0.29 ± 0.01
V_{dil}^{in}	0.00 ± 0.06	0.27 ± 0.04
V_{TCA}	0.86 ± 0.14	0.83 ± 0.05
V_{NT}	0.14 ± 0.05	0.14 ± 0.01
V_x	0.20 ± 0.06	0.43 ± 0.07
V_{dil}^{g}	0.00 ± 0.001	0.07 ± 0.01
V_{GABA}	0.09 ± 0.02	0.07 ± 0.06
V_{PC}	0.000 ± 0.01	0.000 ± 0.01
V_{ex}^{i}	0.021 ± 0.003	0.021 ± 0.003

Table 1. Summary of metabolic fluxes (µmol/g/min, mean ± SD) obtained after modelling and the Monte–Carlo simulation of variance. All metabolic fluxes were described in methods and in Figure 1.