Metabolic imaging of the rat brain using hyperpolarized [1-13C]ketoisocaproate and [1-13C]pyruvate

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Introduction

Branched chain amino acids, and specifically leucine, have unique roles in metabolic regulation, maintenance of glucose homeostasis and translational regulation of protein synthesis. The only sources of this amino acid are through endogenous proteolysis and diet. In rat brain leucine is taken up by the astrocytes and donates the nitrogen for glutamate synthesis via an enzymatic transamination reaction by the branched-chain amino transferases (BCAT) (1). The astrocytes then release ketoisocaproate (KIC) to the extracellular space where it can be taken up by the neurons and converted back to leucine by BCAT. These processes are thought to provide a glutamate-'buffering' mechanism of the excitatory neurotransmitter whereby high intra-neuronal levels and low extracellular levels (in the synaptic cleft) of glutamate are maintained. Histological findings of cytosolic BCAT expression and biodistribution in the rat brain are reported (2).
Exogenous pyruvate is taken up by the neurons and astrocytes. Pyruvate enters the tricarboxylic acid (TCA) cycle or it can be converted to lactate by lactate dehydrogenase (LDH) or the amino acid alanine depending on metabolic need of the cell (3,4).
The aim of our study is to describe regional conversion and biodistribution of hyperpolarized [1-^{13}C]KIC and [1-^{13}C]pyruvate in the normal rat brain with MR spectroscopy and Chemical Shift Imaging (CSI).

Material and method

Five Wistar rats (180-230 g) were anaesthetized with isoflurane. Respiration and temperature were monitored during the scanning session (SA Instruments, USA). Each animal received a 2 mL tail vein injection of 80 mM hyperpolarized [1-^{13}C]KIC (P=15%) and [1-^{13}C]pyruvate (P=30%) (HyperSense polariser, Oxford Instruments or a prototype DNP polariser) in 80 mM TRIS (isotonic) with one hour separation. A 4.7T MR imaging and spectroscopy system (Varian Inc. USA) was used for all experiments. A ^{13}C surface coil in a decoupled 13C/1H volume coil was used (RAPID Biomedical GmbH, Rimpar, Germany). Dynamic ^{13}C spectroscopy from a 10-mm-thick axial slice was acquired (n=2) to determine the optimal CSI time window (20-35 s). The CSI (n=3) (FOV = 30 mm, 16x16 matrix, spiral ordering, FA = 10^{o}) was initiated 20 s after end of injection. Spectral analysis and metabolite image reconstruction was performed in jMRUI (5). Voxels corresponding to different anatomical regions were chosen for each animal based on the proton images to asses to metabolite signal levels.

Results and Discussion

Representative metabolite maps of [1-^{13}C]KIC and [1-^{13}C]pyruvate injection in the same animal are shown in figure 1. Strong [1-^{13}C]KIC and [1-^{13}C]pyruvate signals were observed in the sagittal sinus vein at the top of the images. Examples of voxel position in the sagittal sinus vein (red), cortex (light blue) and hippocampus (dark blue) are given in the proton image.
The CSI signal from leucine is located to the brain tissue. Table 1 displays the mean CSI signal intensities from [1-^{13}C]KIC and [1-^{13}C]pyruvate and their respective metabolites from regions corresponding to the sagittal sinus vein, cortex and hippocampus from three animals.
The ratio of [1-^{13}C]leucine/[1-^{13}C]KIC seems to be higher in the hippocampus compared to cortex.
Substantial ^{13}C-bicarbonate signal was observed and the map show that the metabolite is localized to the brain. The lactate signal was observed in the brain whereas alanine was observed primarily outside the brain in muscle tissue (map not shown) as reported earlier (6).

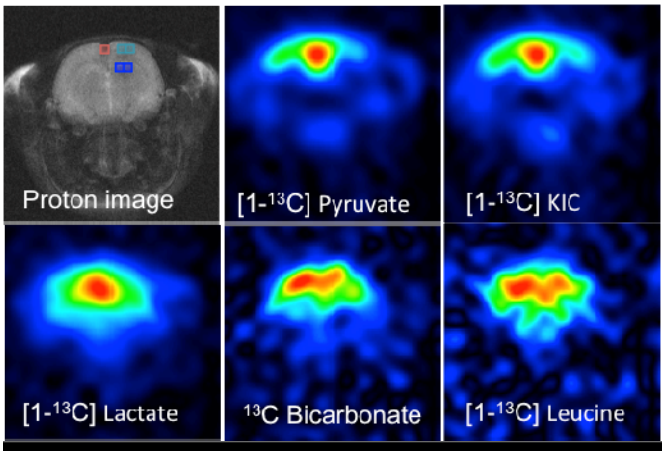

Figure 1: Rat brain proton image with voxel positions in the sagittal sinus vein (red), cortex (light blue) and hippocampus (dark blue) and CSI maps of [1-^{13}C]pyruvate, [1-^{13}C]KIC and their metabolites [1-^{13}C]lactate, ^{13}C bicarbonate and [1-^{13}C] leucine, respectively.

Table 1: The mean CSI signals intensities from [1-^{13}C]KIC, [1-^{13}C]pyruvate, metabolite and metabolite ratios.

Anatomical region/ metabolite signal (A.U.)	KIC±SD	Leu±SD	Leu/KIC±SD	Lac/Pyr±SD	Bic/Pyr±SD
Sagittal sinus vein	84±11	9±3	11±4	78±50	10±3
Cortex	54±1	12±4	23±7	81±15	37±40
Hippocampus	33+3	13+2	40+7	164+72	20+8

Conclusion and Perspectives

In vivo CSI of hyperpolarized [1-^{13}C]KIC metabolism is shown for the first time in a rat brain, demonstrating the potential of studying [1-^{13}C]KIC uptake in the brain and conversion into [1-^{13}C]leucine. Also CSI maps of ^{13}C-bicarbonate are shown for the first time in the normal rat brain. We conclude that the [1-^{13}C]leucine signal reflects the *in vivo* BCAT activity and glutamate pools and the signals from ^{13}C-bicarbonate and [1-^{13}C]lactate reflect TCA activity and LDH activity, respectively.
Both hyperpolarized [1-^{13}C]KIC and [1-^{13}C]pyruvate may be viable substances for brain imaging in e.g. neurodegenerative disease where global or regional metabolism are effected. Further improvement of spatial resolution is required to better delineate substructures regarding metabolite biodistribution.

References: [1] Karlsson et al, Int. J. Cancer 127: 729-736 (2010), [2] Sweatt et al, J. Comp. Neurol 477:360-370 (2004), [3] Navarro and Boveris, Frontiers in Aging Neuroscience 2:34 (2010) [4] Zivkovic and Djuricic, Experientia 15;31(11):1258-60 (1975). [5] Naressi et al, MAGMA. 12:141–152 (2001), [6] Hurd et al, MRM 63:1137-1143 (2010).