Simultaneous dection of metabolism of [2-^{13}C]lactate and uniformly labeled glucose in the brain using in vivo ^{13}C MRS

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Introduction

Although the brain stores very little fuel, it may not use glucose as the only energy source especially under pathological conditions such as hypoxia and ischemia. Lactate can become a significant fuel source and occupy a special position in energy metabolism of the brain, and it may be required energetically to support synaptic function [1, 2]. Measuring lactate in the brain has been instrumental for studying blood flow, consumption of glucose and oxygen, and brain activities [3, 4]. MRS is a powerful tool for noninvasive investigation of brain metabolism and physiology. *In vivo* ^{13}C MRS has been widely used to investigate cerebral metabolism and neurotransmission [5, 6]. ^{13}C isotopomers characterized by the distinct ^{13}C-^{13}C homonuclear splittings could be followed through various stages of the substrate metabolism, thus yielding valuable information which would be difficult to obtain otherwise, which have been exploited in some ex vivo and in vivo studies on the metabolism of certain ^{13}C-labeled chemicals [7]. With the advances in high field *in vivo* MRS technology, this kind of *in vivo* ^{13}C MRS has increasingly been used to observe neurochemical metabolism and especially that involves neuronal-glial interactions in the brain after the infusion of ^{13}C-enriched substrates [8, 9]. In the present study, we show that *in vivo* ^{13}C MRS combined with co-infusion of [$^{13}C_6$]-D-glucose and [2-^{13}C] lactate can be used to simultaneously observe the metabolism of these two different substrates in the carboxylic/amide spectral region.

Methods

Male adult SD rats (193-251 g) fasted 24h were orally intubated and mechanically ventilated with a mixture of ~70% N_2O, 30% O_2 and 1.5% isoflurane and were divided into three groups subjected to intravenous infusions of [$^{13}C_6$]-D-glucose (0.75M) (n = 3), [2-^{13}C]lactate (n = 4) and the co-infusion of [$^{13}C_6$]-D-glucose and [2-^{13}C]lactate (n = 9) respectively. The experiments were performed on an 11.7 Tesla Bruker spectrometer. After coil tuning and matching, MR images were acquired for proper positioning of the animals in the MR scanner. The gradient isocenter was about 0-1 mm posterior to bregma. The left femoral artery was cannulated for periodically sampling arterial blood to monitor blood gases (pO_2, pCO_2), pH, and glucose concentrations using a blood analyzer, and for surveying arterial blood pressure levels. The isolateral (left) vein was also cannulated for intravenous infusion or co-infusion of ^{13}C-labeled chemicals. Normal physiological condition was maintained throughout the experiment (pH ~7.4, pCO_2 ~35 mmHg and pO_2 >100mmHg) and the blood glucose level was maintained at 18.5 ± 1.7 mM.

Results and Discussion

The data processing parameters for all ^{13}C MR spectra were: si =16k, lb = -15, gb = 0.12. Fig. 1 shows the in vivo proton decoupled ^{13}C MRS time course spectra acquired from the rat brain in the 170-186 ppm region with intravenous co-infusion of [2-^{13}C] lactate and [$^{13}C_6$]-D-glucose. Fig.2 shows the comparison of the three in vivo ^{13}C MRS spectra acquired from individual rat brains after intravenous infusions of [2-^{13}C] lactate, [$^{13}C_6$]-D-glucose, and co-infusion of [2-^{13}C] lactate and [$^{13}C_6$]D-glucose, respectively. Glutamate C5 originated from [$^{13}C_6$]-D-glucose appears as a doublet (~182.0 ppm) with a *J* coupling constant of ~51 Hz while glutamate C5 originated from [2-^{13}C]lactate appears as a singlet (182.0 ppm). The large homonuclear ^{13}C-^{13}C coupling constant between an aliphatic carbon and a carboxylic or amide carbon and the lack of interference from other one-bond couplings allow a clean separation of signals originated from different substrates as clearly shown in Figs.1 and 2. At 11.7 Tesla, the chemical shift separation between glutamine C5 and aspartate C4 is coincidentally one half of the one-bond *J* coupling between an aliphatic carbon and a carboxylic or amide carbon. As a result, a pseudo quartet was detected in the 178-179 ppm region, allowing easy separation of contributions to glutamine C5 and aspartate C4 from different ^{13}C-labeled substrates. Since glutamate C1 is widely separated from glutamine C1 and aspartate C1, contributions from different substrates to glutamate C1 are also easily separable. In addition, the features of the spectra acquired during co-infusion matches the sum of the corresponding single-substrate infusion spectra. The time course ^{13}C MRS spectra from the infusion of a single ^{13}C-labeled substrate (either [$^{13}C_6$]-D-glucose or [2-^{13}C] lactate) were not shown here. Comparison of the accumulated ^{13}C MRS spectra with infusion of [2-^{13}C] lactate, [$^{13}C_6$]-D-glucose, and co-infusion of [2-^{13}C] lactate and [$^{13}C_6$]-D-glucose suggests that brains' selection of respiration fuels may be quantitatively measured by this *in vivo* ^{13}C MRS method. In particular, a significant contribution to brain energy metabolism from lactate was seen even at the high blood glucose level of 18.5 ± 1.7 mM, suggesting that lactate is a necessary component of brain energy substrates.

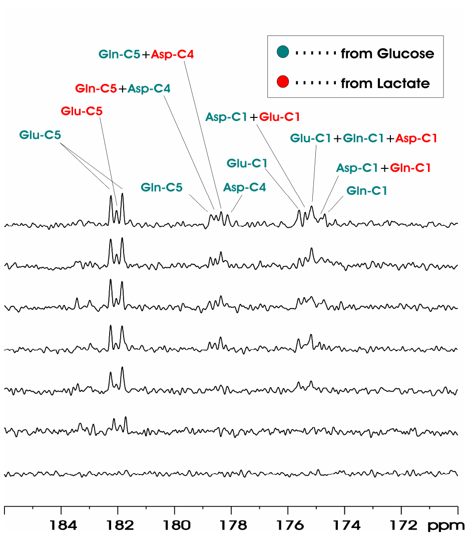

Fig.1. Time-course *in vivo* ^{13}C MRS spectra from an individual rat brain after co-infusion of [2-^{13}C] lactate and [$^{13}C_6$]-D-glucose. Each individual spectrum was averaged for 20 min. **Green**: signals originated from [$^{13}C_6$]-D-glucose; **Red**: signals originated from [2-^{13}C] lactate.

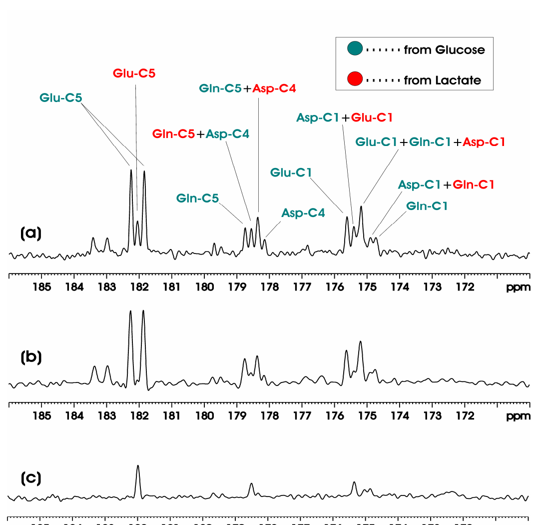

Fig.2. (**a**) Accumulated *in vivo* ^{13}C MRS spectrum during co-infusion of [2-^{13}C] lactate and [$^{13}C_6$]-D-glucose. (**b**) Accumulated in vivo ^{13}C MRS spectrum during infusion of [$^{13}C_6$]-D-glucose only. (**c**) Accumulated in vivo ^{13}C MRS spectrum during infusion of [2-^{13}C]lactate only. All spectra were summed over the 0~180 min interval after the initiation of infusion. **Green**: signals originated from [$^{13}C_6$]-D-glucose; **Red**: signals originated from [2-^{13}C] lactate.

References

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